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**TITLE**                      APPROACHES TOWARDS THE SYNTHESIS OF  
                                    NATURALLY OCCURING TOXIC ALKALOIDS

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**Approaches towards the synthesis of naturally occurring toxic  
alkaloids.**

**By**

**Timothy J. Bond**

**Submitted for the Degree of Doctor of Philosophy**

**The Department Of Chemistry**

**University Of Warwick**

**December 1995**

To all those who have motivated me, from "The Boss", the late Dr. David  
Hutchinson, in the past, to Bronny in the present, and future....

*" Pauling is not an easy man to live up to, but it does me good to try.... "*

Issac Asimov

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Last but not at all least I would like to extend my heartfelt thanks to the late Dr David Hutchinson for putting my feet on the path they now tread.

## Declaration

The work described in this thesis is the original of the author, except where acknowledgement has been made to results and ideas previously mentioned. The work was carried out in The Department of Chemistry, University of Warwick, Coventry over the time October 1991 to Dec 1994.

The work described in this thesis has not been previously submitted for a degree at any other institution.

## Summary

This thesis describes the attempted syntheses of polyamine based peptide toxins (enzymatically) and of 5-mono and 5,8-disubstituted indolizidines (by conventional chemical methods).

Chapter 2 describes the construction of the 5,6 bicyclic skeleton of the indolizidine alkaloids. This was achieved by utilizing *L*-glutamic acid dimethyl ester to form the N-pyrrolo derivative [224], cyclization to the bicyclic compound [214], achieved using acid equivalents, as well as the hydrogenation of [214] to either the alcohol [215] or the fully reduced compound [263] depending on choice of catalyst and pH. Investigations were carried out into the mechanism of hydrogenation of [214] by the attempted synthesis of postulated intermediates [281] and [282]. The results of these syntheses and the submission of the products to our hydrogenation conditions are discussed.

Chapter 3 describes the attempted syntheses of 5-monosubstituted indolizidines from [263] and 5,8-disubstituted indolizidines from [215]. This was undertaken by the activation of the 8-hydroxyl group in [215] and of the alcohol group derived from the ester of [263], followed by attempted substitution. Problems were encountered in both the activation and substitution reactions and these are rationalized in terms of the position of both the alcohol groups  $\beta$  to the nitrogen of the indolizidine skeleton.

Chapter 4 describes attempts towards the differentiation of the two termini of spermidine by various enzymes. The methodology was tested by the chemical protection of the amine functions and attempted enzymatic hydrolysis. The protecting groups chosen were acetyl (Ac) and benzyloxycarbonyl (Cbz) yielding the 1,4-diaminobutane derivatives [378] and [379] and the spermidine derivatives [380] and [381]. The failure of the enzymatic hydrolyses of these compounds is discussed.

## Abbreviations

|                     |                                       |
|---------------------|---------------------------------------|
| [α] <sub>D</sub>    | specific rotation at 589nm            |
| Ac                  | acetate                               |
| bp                  | boiling point                         |
| Bn                  | benzyl                                |
| c                   | concentration (g/100ml)               |
| Cbz                 | benzyloxycarbonyl                     |
| CCL                 | <i>Candida cylindracea</i> lipase     |
| CI                  | chemical ionisation                   |
| <sup>13</sup> C NMR | carbon-13 nuclear magnetic resonance  |
| d                   | day                                   |
| dba                 | dibenzylidene acetone                 |
| DBU                 | 1,8-diazabicyclo[5.4.0]undec-7-ene    |
| DET                 | diethyl tartrate                      |
| DMAP                | 4-dimethylaminopyridine               |
| DMF                 | dimethyl formamide                    |
| Et                  | ethyl                                 |
| F. C.               | flash chromatography                  |
| h                   | hour                                  |
| <sup>1</sup> H NMR  | proton nuclear magnetic resonance     |
| HRMS                | high resolution mass spectrometry     |
| Hz                  | hertz                                 |
| IR                  | infra-red                             |
| J                   | coupling constant                     |
| Me                  | methyl                                |
| min                 | minute                                |
| mp                  | melting point                         |
| MPLC                | medium pressure liquid chromatography |
| Ms                  | mesyl                                 |
| m/z                 | mass to charge ratio                  |
| NCS                 | N-chlorosuccinamide                   |
| PCC                 | pyridinium chlorochromate             |
| Ph                  | phenyl                                |
| Phth                | phthalimido                           |
| PPL                 | pig pancreatic lipase                 |
| <sup>1</sup> Pr     | isopropyl                             |

|                   |   |
|-------------------|---|
| pyr               | pyridine                                  |
| RT                | room temperature                          |
| <sup>t</sup> BDMS | <sup>t</sup> butyldimethylsilyl           |
| THF               | tetrahydrofuran                           |
| TLC               | thin layer chromatography                 |
| TMSOTf            | trimethylsilyl trifluoromethane sulfonate |

# **Introduction. Chapter 1 :**



## 1.1 What is a natural product ?

A natural product is a naturally occurring organic molecule. This definition takes us from relatively simple amino acids to large, complex enzymes such as chymotrypsin. Usually natural products are metabolites, either primary or secondary (Figure 1<sup>1</sup>). The term 'primary metabolite' refers to the products of photosynthetic processes which produce the carboxylic acids (aliphatic amino acids) of the Krebs cycle. These go on to be incorporated into proteins, carbohydrates which form the polysaccharides, fats, nucleotides and the polymers derived from them (DNA, RNA) and proteins. *i.e.* The primary components of the cell and the major players in the dynamic processes of life.

The secondary metabolites (phenolics, terpenoids, steroids and alkaloids) are of limited occurrence and are often characteristic of a particular biological group (family or genus). They are often thought of as waste products, as their function is principally unknown and the organisms can function perfectly well without them (as proved by grafting experiments on plants). These compounds may not be vital for life but, as will be shown, they may play important roles in prolonging life (for example by making the organism distasteful) and perhaps increasing the quality of life (*e.g.* making a mate easier to find).

Indeed, it seems nonsensical to assume that organisms should allocate a large proportion of metabolic resources to the production of compounds which have no use. For example, the ladybird produces the human equivalent of 1.5 kg of the tricyclic *N*-oxide [1],<sup>2</sup> and the honey bee has developed a pheromone gland, the Nassanov gland, for the release of geraniol [2].

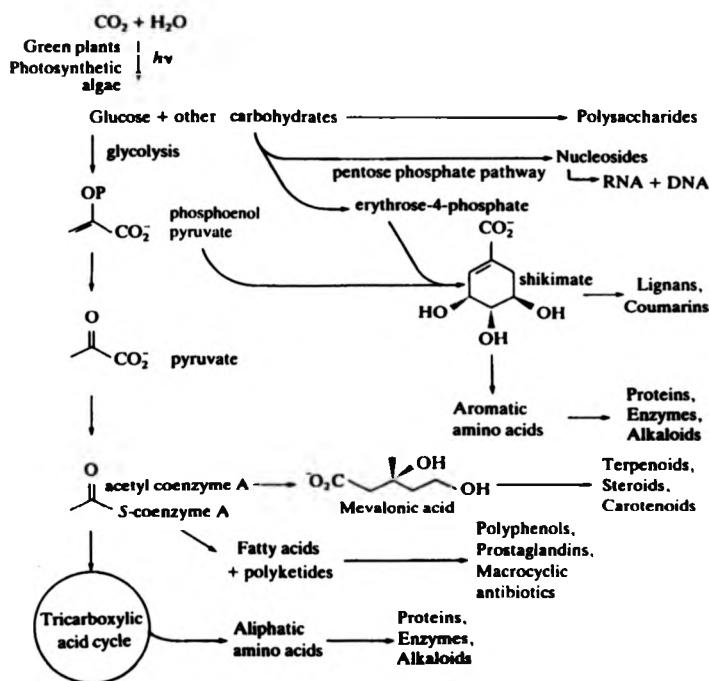
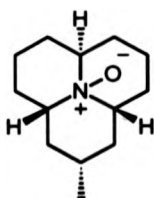
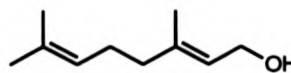


Figure 1. Metabolic production of Natural Products.<sup>1</sup>



[1]



[2]

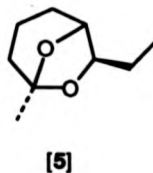
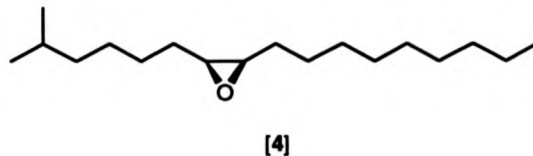
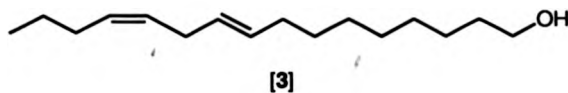
## 1.2 Secondary metabolites as 'effect chemicals'.

Secondary metabolites are 'effect chemicals',<sup>3</sup> viz. compounds that cause a biological effect. However, in the case of most secondary metabolites we have yet to discover the effect, if indeed there is one. Secondary

metabolites from plants and animals can exert strong behaviour control both on members of the same species and of other species. Several areas of chemical control have been proposed:<sup>4</sup>

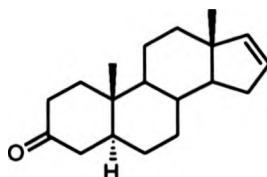
### 1.2.1 Semiochemicals.

Semiochemicals are defined as 'signaling chemicals that carry messages between individuals of the same species'. The messages that these chemicals transmit vary from information as to the position of a food source to sexual attraction. Examples of pheromones used in sexual attraction include the relatively simple moth and beetle pheromones [3], [4] to more complex terpenoids from the same organisms [5], [6].



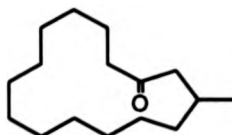
The area of chemical sexual attractants is not limited to insects. Androstenone [7] is a putative human pheromone which is mainly produced by males, though females secrete the steroid to a lesser extent. Indeed, a study by McClintock<sup>5</sup> showed that among women, the higher secretors in an all female dormitory produced synchronous menstrual cycles in the lower

secreting women (synchronous with the higher secretor) to such an extent that all the women in the dormitory attained oestrus at the same time.

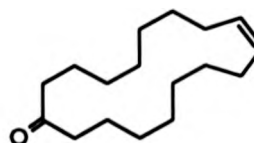


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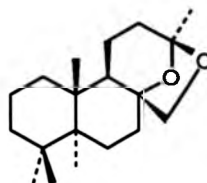
Androstenone is also secreted by male pigs (and truffles, hence the use of sows to dig truffles), a reason why boar meat is not on sale in shops (due to its unpleasant smell upon cooking). Androstenone is also used in aerosol form to arouse sows as well as in certain types of aftershave which are reported to increase sex appeal. There are also age-old links between secondary metabolites and sexual arousal. Humans have daubed themselves with the secretions of the penile sheath of the musk deer, containing muscone [8], those of the scent glands of the civet cat, containing civetone [9], secretions from sperm whales (ambergris [10]) and the odours of various flowers and spices in the complex mixtures of today's perfumes to increase their attractiveness to the opposite sex.



[8]



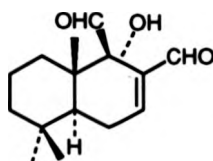
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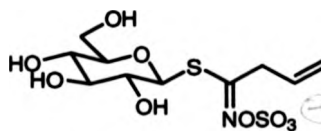
[10]

### 1.2.2 Feedants, antifeedants, repellents and toxins.

Many plants secrete chemicals that make them unpleasant to taste and sometimes even poisonous to the insects and higher organisms that feed on them. Warburganal [11] has been isolated<sup>6</sup> from the East African tree *Warburgia stuhlmannii* and has been found to have an antifeedant effect upon the larvae of the army worm. However, such compounds do not have a universal effect. Warburganal has no effect on the locust and, in another example, the glycosinate sinigrin [12], though it repels most insects, is an attractant to the cabbage butterfly (*Pieris brassicae*).

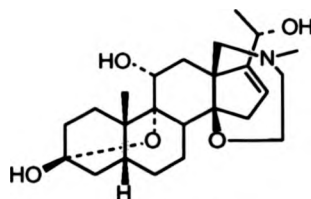


[11]

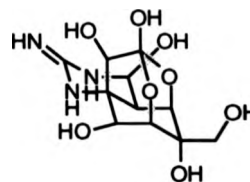


[12]

*Danus* monarch butterflies have adapted to accumulate toxic cardenolides from food sources while caterpillars which repel birds from the adults.<sup>4</sup> Toxins are widespread in the natural world and many plants and animals produce these compounds. Two of these are batrachotoxin [13] from the skin of a Colombian frog and tetrodotoxin [14] from the Japanese puffer fish.



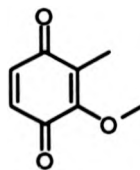
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[14]

### 1.2.3 Defense and alarm.

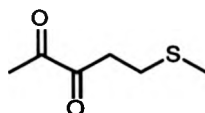
Natural products secreted at the time of, or stored and released upon, attack in defense are many and varied as in the quinone [15] exuded by the millipede *Nurceus gardanus* or a mixture of simple benzoquinines projected by the bombardier beetle *Brachyrus crepitans*.<sup>7</sup> The best known of these defenses is probably the mercaptan [16] released from the anal glands of the skunk when startled or attacked. The hyena also releases an anal exudate [17] under stress. Charles Butler noted in 1609 that bees could be attracted and enraged to sting by the presence of a single bee sting.<sup>3</sup> We now know that this is in response to isoamyl acetate [18], an alarm pheromone released when a bee's abdomen is ripped (releasing the sting).



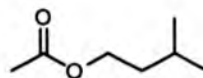
[15]



[16]



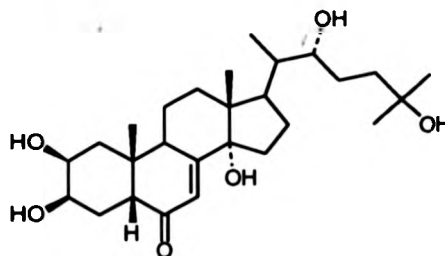
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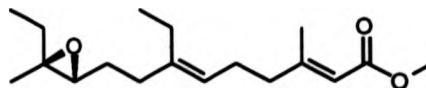
[18]

#### 1.2.4 Development and growth.

The steroid ecdysone [19] stimulates metamorphosis. The so-called juvenile hormone [20] exerts the opposite effect of preventing metamorphosis of insects and thus hampering the development into adults. These two compounds have obvious potential in cases where controlling the life cycle of an insect would be of use, for example in pesticides.



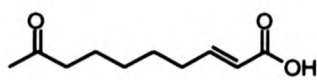
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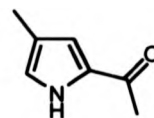
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### 1.2.5 Social behaviour.

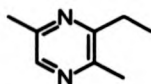
The 'social insects', viz. termites, some bees and wasps and the ants, are the greatest users of semiochemicals. The social organisation of their hives and nests means that the individuals have more need to communicate. The so-called queen substance [21] (9-oxo-2-decenoic acid) produced by the honey bee queen stabilises swarms, controls queen-cell building and attracts drones to mate. A whole series of semiochemicals have been found e.g. [22]-[26] which are used by ants to mark trails which lead fellow workers both to food sources and back to the nest.<sup>3</sup>



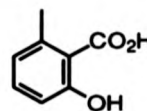
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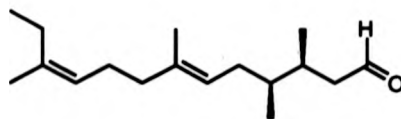
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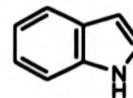
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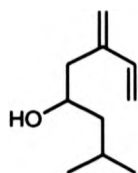


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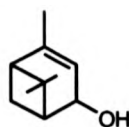
Bees use geraniol [2] as a marker pheromone to tag sources of food, water and the nest. Bark beetle pheromones are a mixture of compounds e.g. [27]-[29] that are aggregation pheromones for both sexes.<sup>8</sup> These are of particular



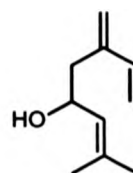
interest in the treatment of Dutch elm disease which is carried by these beetles.



[27]



[28]



[29]

## 1.3 History of natural products.

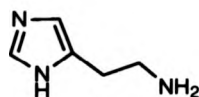
### 1.3.1 Ancient history.

Natural products, and their sources have long fascinated man with their intriguing biological activities. The emperor Shen Nung recommended the laxative properties of rhubarb in his Pharmacopoeia (*ca* 2700 BC) and Homer and Virgil both mention the use of 'potions' derived from natural sources to treat illness and sometimes to induce it. Fragrances and spices, all of whose active ingredients are natural products have long been extolled. This is illustrated in the 'Song of Songs' from Ecclesiastics II,<sup>9</sup> apparently written by King Solomon to the Queen of Sheba, showing the Israelites' fascination with natural products dating back to 1000 BC.

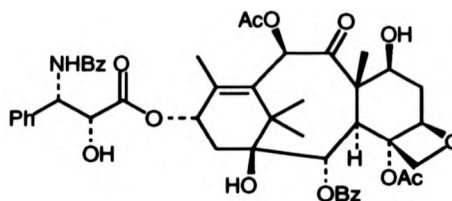
### 1.3.2 Modern history.

Scientists are still fascinated by the varied biological activities of natural products. John Ray in 1690 commented upon attraction of male moths to a caged female<sup>10</sup> and today whole teams of scientists are engaged in the extraction, identification and testing of compounds from natural sources.

The arena of natural products provides a range of synthetic targets unparalleled in any other area of chemistry. This is amply illustrated by looking at the alkaloids, which are the most structurally diverse class of secondary metabolites. Over 5000 compounds are known, ranging from the structurally simple histamine [30] to the complex structure of taxol [31]. Taxol, though a relatively small molecule, has eleven chiral centers.



[30]



[31]

Although many alkaloids were isolated and characterised decades ago, new and interesting alkaloids, some structurally related, others being the first members of new classes, are continuously being discovered in great numbers. Thus, the synthetic challenge goes on.

## 1.4 The need to synthesise natural products.

Nature has developed complex synthetic routes to primary and secondary metabolites. We could argue that with the use of the most structurally demanding and efficient catalysts known, *viz.* enzymes, nature will synthesise natural products better than us. We have alluded to the intellectual challenge in the previous section but there are other reasons.

The first synthesis of a natural product, *viz.* Wöhler's urea,<sup>11</sup> was one of the important steps in refuting the doctrine of vitalism, *i.e.* the theological

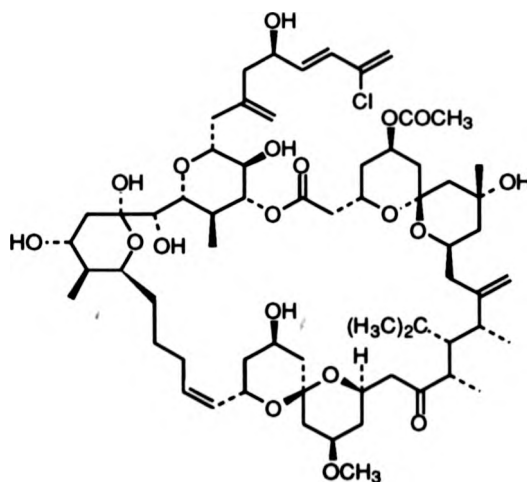
idea of a 'mysterious force' pervading all living matter and differentiating it from non-living. This proved that compounds produced from living things were in no way different than those produced chemically in the lab. With our present knowledge of chemistry, biochemistry and biology we need no further proof that natural and 'unnatural' (produced in the lab) compounds are the same, with the same activity in biological systems. So this reason for natural product synthesis belongs to the past.

Over 60% of the world's population are estimated to use, at some point in their lives, medicinal chemicals derived from animal and plant sources. Over half of all U.S. prescriptions fall into this category. Most of the remaining half are synthetic compounds based on chemical modifications of natural substances. Approximately 120 drugs of known structure are still extracted from their natural sources for use. Opium, in use for at least 5000 years, and cocaine, used for *ca.* 2000 years, would both fall into this category. Ancient Chinese herbalism has been the source of many of these drugs and, although the Chinese market consumes some of these, most of the 120 are used in western medicine.

As mentioned, many of the drugs on the market are analogues of natural products. Chemical modifications can produce new drugs with superior activity against the target illness. So, the synthesis of natural products is of great importance to provide 'lead' compounds, suggesting the functionality needed to achieve an active end product.

Secondary metabolites are not, as a rule, produced in large quantities. For example, spongistatin 1 [32], a potent antineoplastic agent isolated from the south west African marine sponge *Spirastrella spinispirulifera*, has recently been isolated. Just 1.8 mg was obtained from 2409 Kg of wet sponge.<sup>12</sup>

Taxol [31], isolated from the bark of the pacific yew in minor amounts,<sup>13</sup> also it is only found in this species and in a specific geographic location. The pacific yew has a very slow growth rate. Thus to supply taxol for widespread clinical trials would mean the extinction of the species and no more natural taxol. So, the synthesis of natural products has yet another purpose. That is to provide pharmaceutically interesting compounds in quantities sufficient for evaluation of activity.



[32]

## 1.5 The desirable properties of a synthesis.

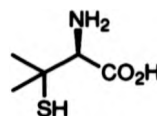
All syntheses have desirable properties, that isolation and separation of the product from starting materials and by-products is feasible, high yields, few synthetic steps *etc.* A number of these properties are particularly desirable in the synthesis of a natural product as the product is to be as close to that from the natural source as possible. Also of consideration is the use of the synthesised natural product as a lead compound for the synthesis of

analogues, and that enough be produced for practical purposes. In particular, we can identify two important considerations:

### 1.5.1 Enantiomeric purity of products.

Chirality, if this meant simply that two enantiomers rotated plane polarised light in equal and opposite directions would have little bearing on synthetic organic chemistry. However, enantiomers only have identical chemical and physical properties in the absence of an external chiral influence and this is where the problem becomes more complex. Life is chiral as most of the important building blocks of biological macromolecules are chiral *e.g.* amino acids and sugars.

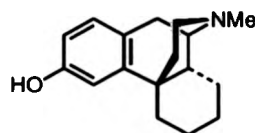
If both enantiomers are active there is no guarantee that both will be equally active. Indeed, they may have entirely different effects. For example, (*R*)-penicillamine [33] is an antidote for lead and mercury poisoning whereas the enantiomer (*S*)-penicillamine, causes the optic nerve to atrophy leading to blindness.



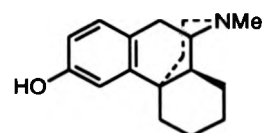
[33]

The American FDA (Federal Drug Administration) will not now allow the marketing of a racemate to humans unless the enantiomer which is not needed is proven to be inert and cause no side effects. It is possible that both enantiomers may have a favourable, though different, effect. The alkaloid (-)-levorphanol [34] is a powerful narcotic analgesic exhibiting an activity five to

six times that of morphine. Its enantiomer, (+)-dextrorphan [35], is a cough suppressant.



[34]

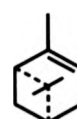


[35]

As a footnote to this discussion it should be stated that not all natural products are enantiomerically pure. For example the crude terpene  $\alpha$ -pinene is actually a mixture of 92% [36] and 4% [37] when isolated from pine resin.



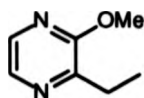
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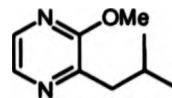
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### 1.5.2 Analogue synthesis.

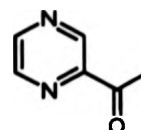
Often chemically closely related structures can have quite different properties. The methyl ether [38] has the distinctive raw potato odour, the branched ether [39] smells like green peppers and the methyl ketone [40] of popcorn.<sup>14</sup>



[38]

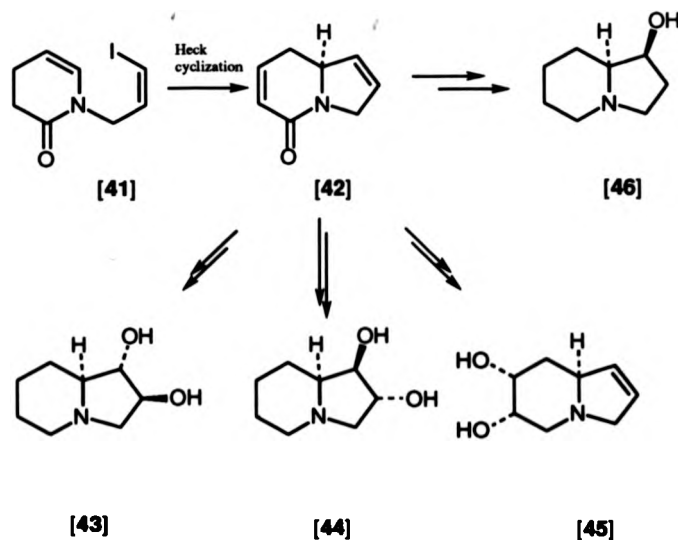


[39]



[40]

So, small structural modifications and functional group changes have a large effect on the physiological action of some of these compounds. Some syntheses are designed to contain a late-stage common intermediate, one which can undergo a variety of reactions to produce a variety of related products and thus be of greater synthetic utility than a more linear approach. An example of this comes from Nukui *et al.*<sup>15</sup> who undertook the asymmetric Heck-type cyclization of [41] to give the unsaturated bicyclic compound [42], asymmetry being induced through a chiral palladium species in the presence of silver carbonate. From this they have achieved the synthesis of the related lentiginosine [43] and 1,2-dipilentiginosine [44] as well as the indolizidines [45] and [46] (Scheme 1). They propose that with further functionalisation many other related natural and unnatural products can be synthesised from this common core.

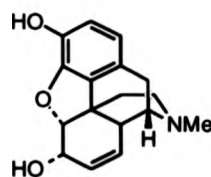


Scheme 1

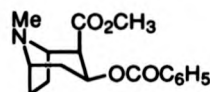
## 1.6 The Alkaloids.

### 1.6.1 Introduction.

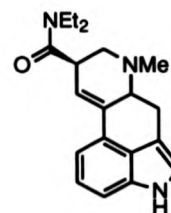
The alkaloids are probably the group of compounds which have been used more than any others in the pursuit of pleasure and as deadly poisons and sedatives. Examples of euphorants, psychedelics and stimulants include morphine [47] from *Papaver somniferum*, cocaine [48] from *Erythroxylon coca*, lysergic acid [49] and derivatives, in *Claviceps purpurea*, and mescaline [50], in the indian peote cactus *Lophophora williamsii*, used by the American indian shamen to ascend to the astral plane to communicate with their gods. The Mayan priesthood used the Mexican mushroom *Psilocybe mexicana* (containing psilocybin [51]) with the same goal.



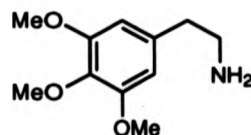
[47]



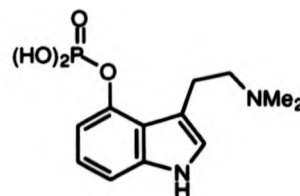
[48]



[49]



[50]



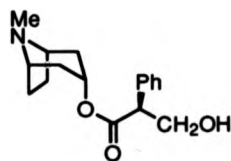
[51]

Plant extracts have been used since ancient times to induce death and disability. Cleopatra, before deciding on the venom of the asp as the vehicle

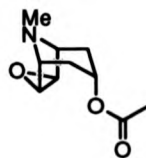


for her own demise, conducted tests on slaves using henbane (*Hycoscyamus niger*), deadly nightshade (*Atropa belladonna*), and *Strychnos nux vomica*. All were quick but painful, *Strychnos nux vomica* having the added disadvantage of leaving the victim's face contorted in death. *Atropa belladonna* berries (containing atropine [52]) were used by Agrippina, wife of the Roman emperor Claudius, to murder him, the juice being injected into the fruit of his personal fig tree.

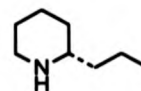
Hycosine [53], from the mandrake (*Mandrogora officinium*) was a favorite of the renaissance poisoner Cesare Borgia. Other plants of the *Solenaceae* family (which includes the tomato and the potato) contain hycosine [53] as a major constituent. This active ingredient has been utilised by many, for example by Dr. Crippen as a poison, by suckling Colombian mothers who smeared the extract on their nipples as an infanticide agent and by 16<sup>th</sup> century Hindu prostitutes who used more dilute extracts to subdue their clients, thus curbing their carnal appetites.



[52]

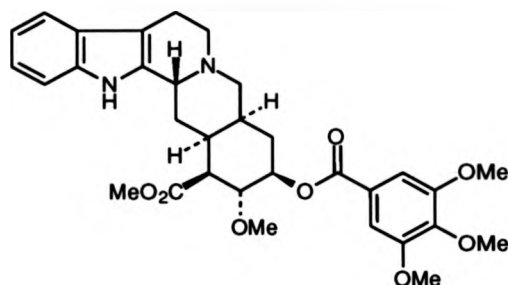


[53]



[54]

Perhaps the most infamous death by alkaloid was that of Socrates. Coniine [54] from *Conium maculatum* was a commonly used poison for state executions and indeed Socrates' death was induced with this drug. Modern medicine harnesses many of these tools of destruction. Atropine [52] is used in eye surgery, morphine [47] as a pain killer and reserpine [55] as a tranquilliser and many modern day drugs contain similar compounds.



[55]

The traditional definition of an alkaloid, first put forward by W. Meissner in 1879, encompassed "nitrogenous compounds of complex molecular structure and significant pharmacological activity confined to the plant kingdom". The isolation, in recent years, of alkaloids from insect, amphibian and even mammalian sources has rendered this classification obsolete. Pelletier's more recent definition, proposed in 1982, is, "cyclic nitrogen-containing molecules, which are true secondary metabolites". A further stipulation, that the nitrogen atom must have a negative oxidation state excludes nitro and nitroso compounds, though both these groups may be present in the side chains.

### 1.6.2 Classification of Alkaloids.

Most compounds covered by Pelletier's definition can be constructed from building blocks of the shikimic acid, polyketide or mevalonic acid pathways in conjunction with an amino acid which determines the character of the alkaloid and thus its classification. Ornithine [56] gives rise to, for example hygrine [57], retronecine [58] and nicotine [59]. Lysine [60] is the precursor to isopelletierine [61] and lupine [62] (Figure 2).

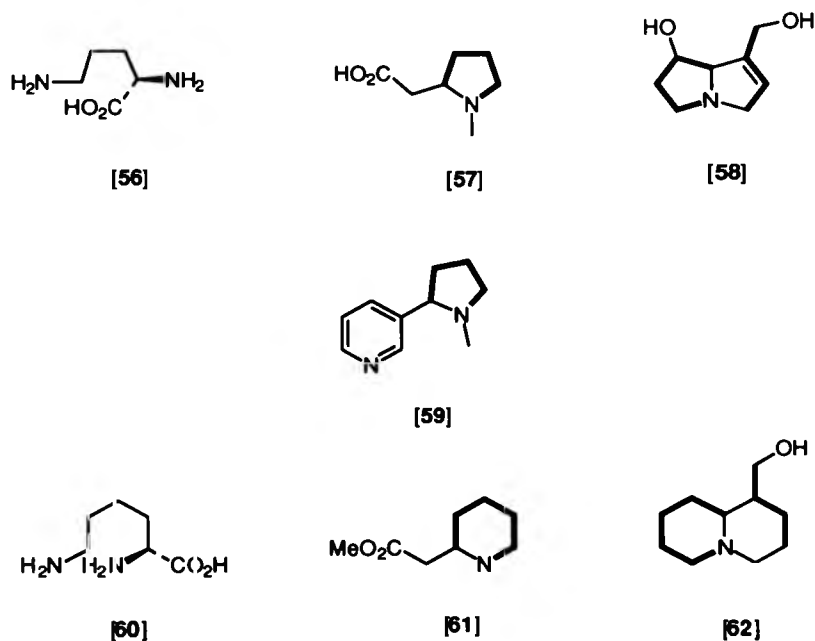
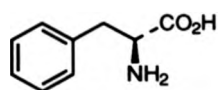


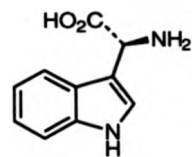
Figure 2. Alkaloids derived from ornithine and lysine.

The aromatic amino acids phenylalanine [63] and tryptophan [64] give rise to alkaloids such as morphine [47] and strychnine [65] (Figure 3)<sup>1</sup>. There is also a class of 'pseudoalkaloids', those not derived from amino acids but from a polyketide or terpenoid, the nitrogen atom coming ultimately from ammonia. Coniine [54] is an example of a pseudoalkaloid.

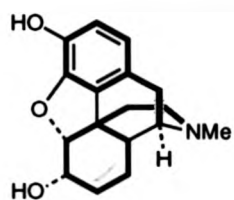
Alkaloids from amphibian sources are structurally related to the pyrrolizidines, quinolizidines and other piperidine-type alkaloids. These are derived from ornithine and lysine and will be discussed in Chapter 2. The other classes will not be discussed in any further depth as there are good reviews of their occurrence, biosynthesis and synthesis.<sup>1,4,14</sup>



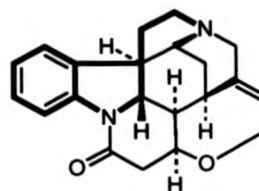
[63]



[64]



[47]



[65]

**Figure 3.** Alkaloids derived from phenylalanine and tyrosine

## **Frog toxins. Chapter: 2**

## 2.1 Amphibian Alkaloids.

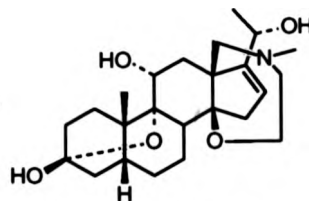
In recent years, there has been much interest in the biological evaluation of the natural products found in the skin extracts of neotropical poison frogs.<sup>16</sup> The skin extracts contain four major classes of compounds with a range of toxicity, some merely noxious, some highly toxic. These are biogenic amines, peptides, bufodienolides (bufogenins) and alkaloids. There are more than 200 'dendrobatid' alkaloids, so-called as most are unique to the neotropical poison frogs of the genera *Dendrobates* and *Phylllobates* (*Dendrobatidae*), of diverse structure and biological activity. These compounds can be divided into major and minor classes, these terms pertaining to the number of alkaloids in each class and/or ubiquity among the species. Some of the classes are named after the species from which they were first isolated. For example the pumiliotoxins A, B and C were isolated from the Panamanian frog *D. pumilio* and a Colombian frog, *D. histrionicus* afforded the histrionicotoxins.

The dendrobatid alkaloids are secreted in such small amounts that structural determination has required the sacrifice of many frogs. Even then, most of the structural determination was carried out using TLC and GC-MS on the alkaloid residues.<sup>17,18,19</sup> Very few were isolated in sufficient amounts for NMR analysis. After TLC analysis and sometimes preparative TLC, GC analysis was undertaken in four steps. Firstly GC provided a quantitative profile of alkaloid components.<sup>18</sup> This was followed by GC-NH<sub>3</sub>CIMS with computer assistance to provide the number, elution sequence and parent ions of the alkaloids present. Repetition of GC-CIMS using deuterioammonia showed the number of exchangeable hydrogens (OH and secondary amines).

Finally GC-EIMS was carried out. Usually collation and analysis of this data allowed characterisation and identification of the individual alkaloids.

### 2.1.1 Batrachotoxins.

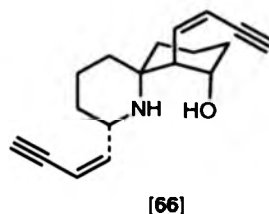
The batrachotoxin class contains few compounds but they are highly toxic steroidal alkaloids, such as Batrachotoxin A [13] occurring only in species *phyllobates*. They were the first of the dendrobatid alkaloids to be isolated and characterised.<sup>20,21</sup> The six known batrachotoxins occur in relatively large amounts in the skin extracts of the *phyllobates* family. *P. terribilis* secretes up to 800 µg per frog as a mixture of these.



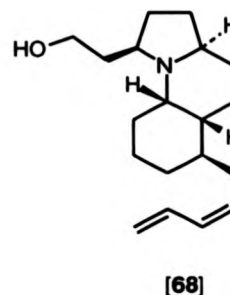
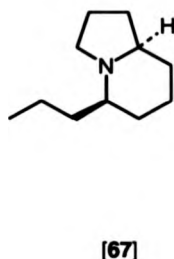
[13]

### 2.1.2 Histrionicotoxins.

Histrionicotoxins, for example Histrionicotoxin A [66], were first isolated from *Dendrobates histrionicus*.<sup>22</sup> These relatively non-toxic azaspiro[5.5]undecanols have two exchangeable protons (OH and NH). The 16 known examples differ in the length and degree of unsaturation in the two side chains. The stereochemistry of the alkene functionality is usually *cis* (where determined).



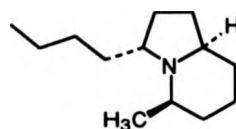
### 2.1.3 Indolizidines.



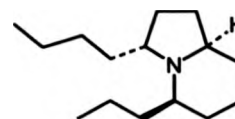
The dendrobatid alkaloids include a large variety of indolizidines from the relatively simple mono-substituted 167B [67] to the complex gephyrotoxin 289B [68]. Gephyrotoxins contain the indolizidine skeleton with additional functionality which characterises this class, though this is an older classification. Simple indolizidines, such as 195B [69], 223AB [70], 239AB [71], 239CD [72], were originally classified as gephyrotoxins but are now referred to, as are all the dendrobatid alkaloids, by the system proposed by Daly *et al.*<sup>18</sup> This system assigns all the dendrobatid alkaloids a number which is simply the molecular weight. If more than one alkaloid has the same molecular weight an identifying letter A, B *etc.* is assigned. For those with a close structural relationship prime and double prime designations are added to clarify this point. Indolizidines with the double letter designation *e.g.* 223AB [70] were originally assigned separate structures based on incorrect spectral



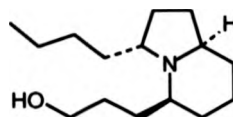
assignments.<sup>18</sup> When the GC results were rationalised the double letter designation came into use<sup>16</sup> to indicate both structures were in fact the same.



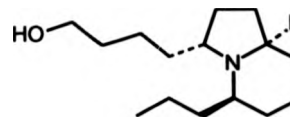
[69]



[70]

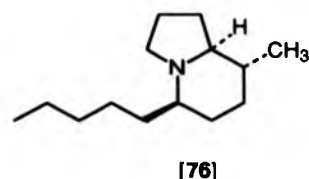
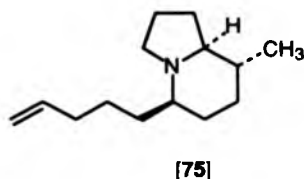
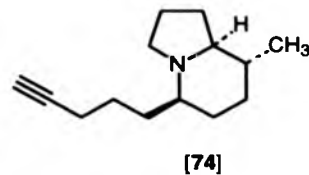
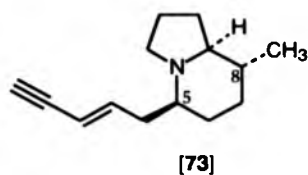


[71]



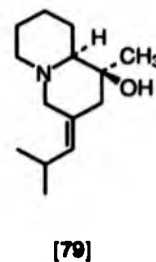
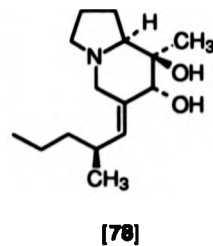
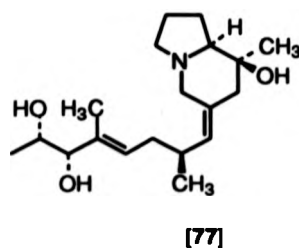
[72]

The indolizidine class may be subdivided into 3,5-disubstituted and 5,8-disubstituted indolizidines. The 3,5-disubstituted indolizidines were the first to be discovered, 195B [69], 223AB [70], 239AB [71], 239CD [72], being those isolated in sufficient quantities to be assigned unambiguously by NMR.<sup>19,23</sup> A series of congeneric 8-methylindolizidines, 203A [73], 205A [74], 207A [75], 209A [76] have been found, their structures differing only in the degree of unsaturation in the 5-carbon side chain.<sup>19,24</sup> The dendrobatid alkaloids include many simple bicyclic alkaloids containing a tertiary nitrogen and usually a piperidine ring. Many of these are assumed to be indolizidines when the tentative structures are assigned. Indeed 207A [75], originally of unidentified structure<sup>17</sup> was recently shown to be 5-(pent-5-enyl)-8-methyl indolizidine, the first of this 5,8-disubstituted class. There are many members of this class and all bicyclic tertiary amine alkaloids with a base peak at  $m/z$  138 are put in this class.



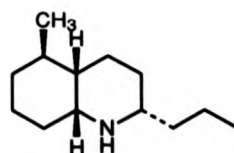
#### 2.1.4 Pumiliotoxin-A class.

Pumiliotoxin-A [77], was isolated in 1967 from the Panamanian frog *Dendrobates pumilio*,<sup>25</sup> but the structure was not assigned until 1980.<sup>26</sup> The pumiliotoxins could also be thought of as indolizidines, as they have the standard 5,6 bicyclic structure. The characteristic additional features of pumiliotoxins are a quaternary 8 position and an exocyclic double bond at C-6 (e.g. 323A [77]). The allopumiliotoxins (e.g. 253A [78]) have an additional hydroxyl function at C-7 and homopumiliotoxin 223G [79] has, rather than the indolizidine skeleton, a quinolizidine ring system.

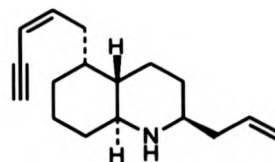


### 2.1.5 Decahydroquinolines

The older 'pumiliotoxin-C' class designation has been superseded by the term decahydroquinolines. Pumiliotoxin C 195A [80] was the first member of this class to be isolated.<sup>27</sup> Interestingly, both *cis* and *trans* decahydroquinolines [80], [81] are known. They normally occur together with the histrionicotoxins in the species of *dendrobates* and *phyllobates*. This led to the postulation that they represent members of a deoxyhistrionicotoxin class, though with no proof.<sup>17</sup>



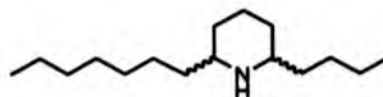
[80]



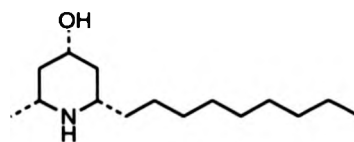
[81]

### 2.1.6 The minor alkaloids.

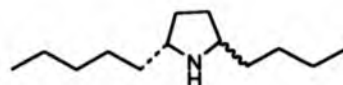
The seven 'minor' classes of dendrobatid alkaloids are referred to as such due either to limited variety within the class or limited taxonomic distribution. They normally occur as trace alkaloids, often in only one or two species or even only in one or two populations of the same species. For instance the pyrrolidine alkaloid 239I [82] was only found in a single specimen of *D. trivittatus* as was 241D [83], recently isolated from *D. speciosus*.



[82]

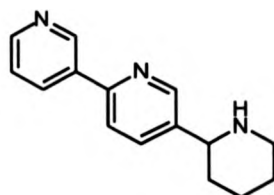


[83]

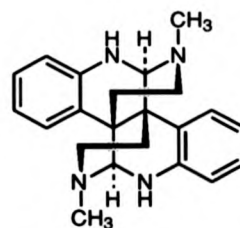


[84]

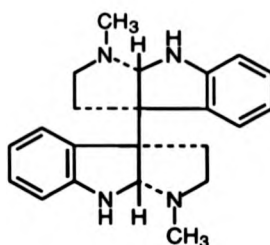
A further reason for their 'minor alkaloid' classification is that these compounds may not be secondary metabolites of the frogs, but assimilated (perhaps with some subsequent metabolism ) from food sources.<sup>28</sup> Possible precursors to alkaloids found in dendrobatid frogs are found both in insect and plant species. Both 2,6-cis and 2,6-trans- [83] have been found in South African fire ants of the genus *Solenopsis*,<sup>29</sup> and *trans*-[84] has been identified from *S. punctaticeps* and the Pharaoh ant *Monomorium pharaonis*.<sup>30</sup> Noranabasamine [85] (pyridyl-piperidine class) corresponds to a *N*-desmethyl analogue of anabasamine, a plant alkaloid, and the two isomeric alkaloids calycanthine [86] and chimonanthine [87] (though opposite enantiomers) are known from the North American plant genus *Calycanthus* (*Calycanthaceae*). Another alkaloid which may be assimilated and perhaps metabolised from dietary sources is the azatricyclododecene 205B [88], reminiscent of alkaloids from the ladybird beetles of the family *Collienllidae*. For further discussion of the biogenesis of the dendrobatid alkaloids see 2.2.1.



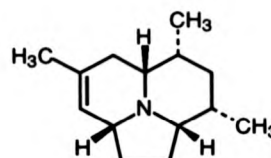
[85]



[86]



[87]



[88]

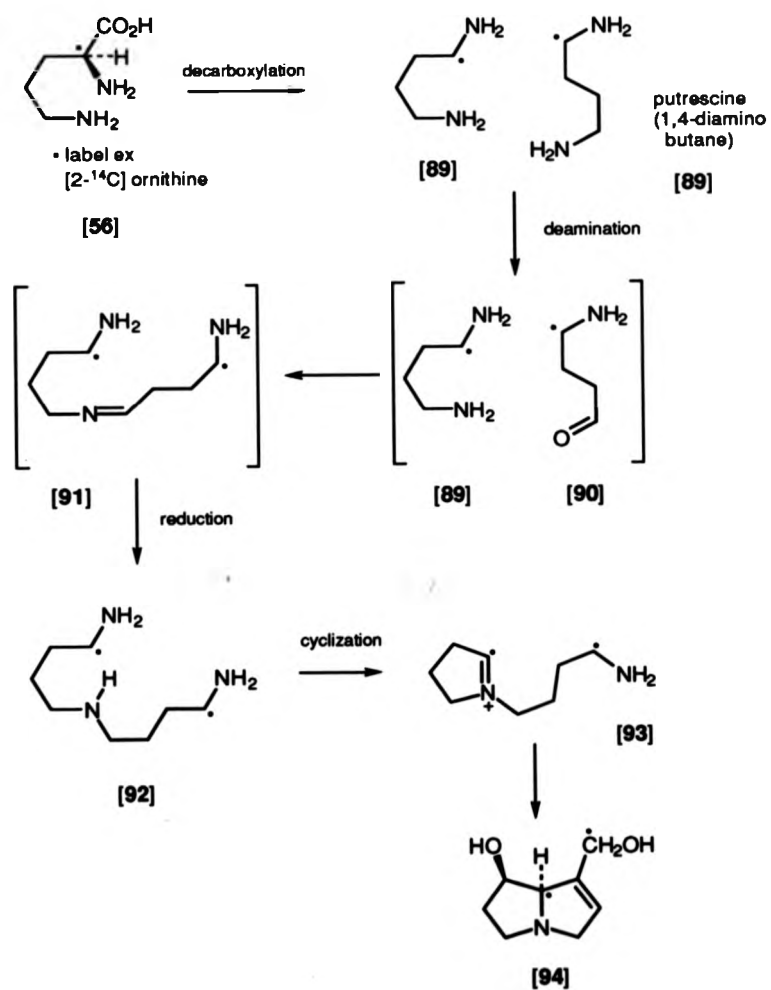
## 2.2 Biology.

### 2.2.1 Biological origins.

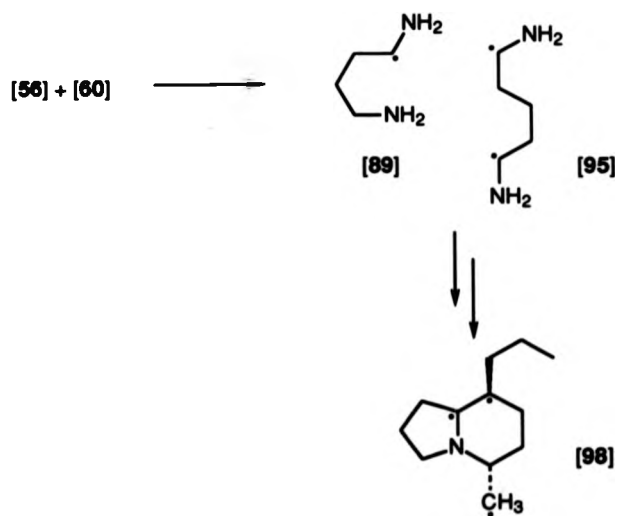
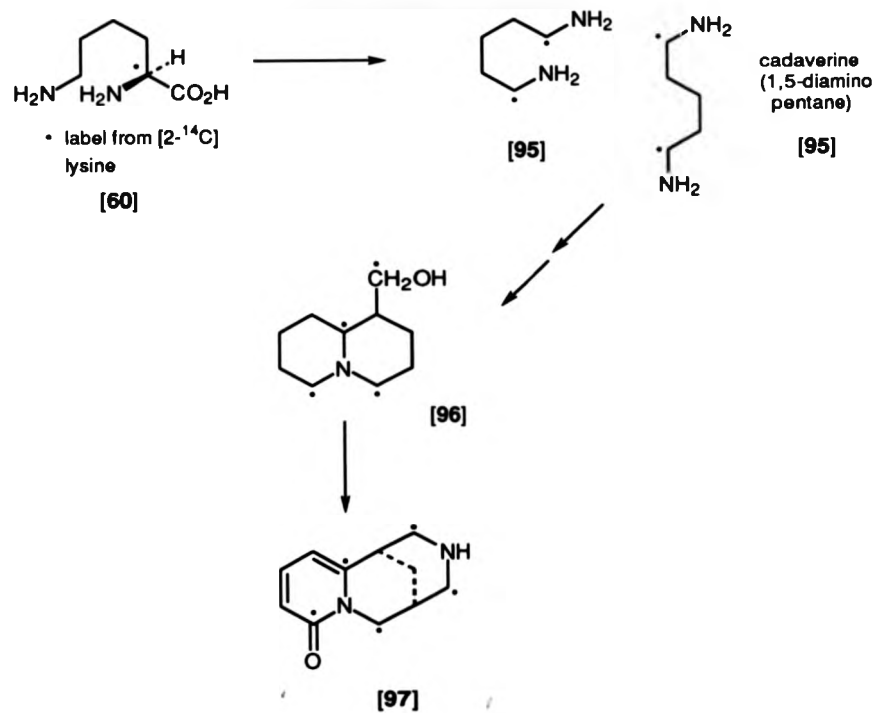
The amphibia (frogs, toads and salamanders) produce a wide variety of secondary metabolites including peptides, biogenic amines, bufodienolides and over a dozen classes of alkaloids. The wide structural variety of the dendrobatid alkaloids leads to two conclusions: a) the amphibia have evolved complex biosynthetic pathways to produce these alkaloids, or b) precursors or even the alkaloids themselves have a dietary origin. Biosynthetic studies to date have not shed much light on the subject. *In vivo* studies found no detectable incorporation of radioactive acetate, mevalonate or cholesterol into the alkaloid fractions of *D. pumilio* or *P. aurotino*. Similarly, no radioactive serine was incorporated into the batrachotoxin alkaloids of *P. aurotaenia*. The

radioactive acetate and mevalonate were incorporated, instead, into skin cholesterol.<sup>31</sup> Similar results were obtained in the investigation of tetrodotoxin (TTX) [14] biosynthesis. Radioactive acetate, arginine and citronelline were incorporated into cholesterol, amines and amino acids but not TTX in the californian newts of the genus *Taricha*.<sup>32</sup> A cautionary note should be added here that this evidence could point to very slow alkaloid synthesis, with no incorporation on the time scale of these experiments.<sup>31</sup> Since there is the suggestion that acetate, mevalonate and cholesterol are not involved, an equally valid suggestion may be put forward that the biosynthetic pathway is similar to that of the related pyrrolizidines and quinolizidines. These alkaloids are derived from ornithine and lysine. There are in both these cases two possible starting materials. Pyrrolizidines *e.g.* retonicine [94], may be formed from ornithine [56] or putrescine [89] (Scheme 2) and quinolizidines *e.g.* cytisine [97], from lysine [60] or cadaverine [95] (Scheme 3).<sup>1</sup>

Biosynthesis takes place by the coupling of two putrescine or two cadaverine units to yield, respectively, a 5,5 or a 6,6 bicyclic alkaloid. If one putrescine and one cadaverine unit were to be joined a 5,6-bicyclic alkaloid might result (Scheme 4). This could be determined by feeding experiments with labeled [89] and [95]. The predicted labeling pattern is shown in [98].



Scheme 2





The other option, that either the alkaloids themselves or precursors to them are accumulated from dietary sources, is supported by the fact that toxic alkaloids are all but absent in the eggs and larvae of the dendrobatid frogs and the related TTX is absent in hatchery-raised puffer fish.<sup>33</sup> Indeed, second generation laboratory reared *P. terribilis* have no detectable levels of batrachotoxin, though the alkaloid profiles of wild-caught adult frogs in captivity remains relatively constant fed on *Drosophila* for up to six years. The absence from the eggs and larvae could be attributable to the non-development of the 'poison glands' at this stage or to the slow turn-over of alkaloid production. The lack of alkaloid production in lab reared specimens suggests a dietary origin of the alkaloids in their wild cousins, but this is put into doubt by the consistent alkaloid profiles of wild-caught adults.

The dietary origins of these alkaloids is further put in question when the apparently random phylogenic occurrence of TTXs is considered. TTXs are found in species as diverse as marine snails,<sup>34</sup> flatworms,<sup>35</sup> starfish,<sup>36</sup> the blue-ringed octopus<sup>37</sup> and puffer fish<sup>33</sup> as well as in salamanders<sup>38</sup> and frogs.<sup>39,40</sup> This occurrence in marine, freshwater aquatic and terrestrial species suggests that a dietary origin is unlikely, though bacterial production may be involved.<sup>41</sup> Further support for the dietary origins of some of the alkaloids comes from the isolation of morphine, a well known plant alkaloid from the skin extracts of the toad *B. marinus*.<sup>42</sup>

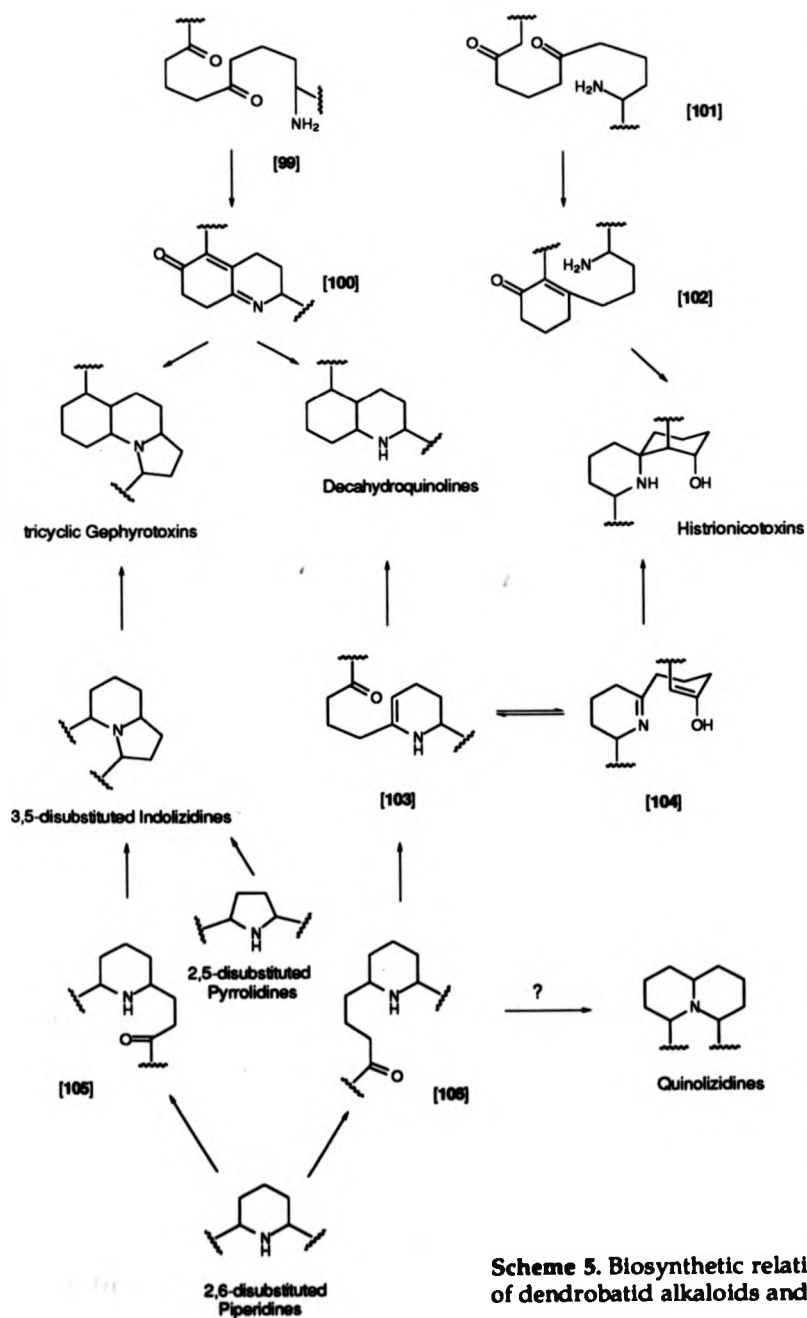
2,6-Disubstituted piperidines have been suggested as possible precursors to histrionicotoxins, gephyrotoxins, indolizidines and decahydroquinolines.<sup>43</sup> The recently discovered 2,5-disubstituted pyrrolidines have been suggested as precursors to dendrobatid alkaloids containing the indolizidine moiety, e.g. the pumiliotoxin A class.<sup>16</sup> However, the existence of, for example, 5,8-

disubstituted indolizidines cannot be rationalised using either 2,6-disubstituted piperidines or 2,5-disubstituted pyrrolidines as precursors. These suggestions seem unreasonable considering the limited occurrence of these alkaloids (section 2.1.6). If these were the precursors, significant amounts should have been found in the skin extracts of most of the species investigated, not just the three examples known to date. Further work is needed in this area before any conclusions can be drawn.

### 2.2.2 Biological roles.

Three species of South American *Phylllobates* are used by the Emberá and Noanamá Chocó indians for poisoning blowgun darts. These species were chosen due to their high toxicity (we now know this is due to their high content of batrachotoxin [13]), which caused locomotor difficulties, convulsions and death. Frogs of the genus *Dendrobates* have evolved simpler, less toxic alkaloids. These produce, in mice, reactions as diverse as Straub tail, penile erection, prostation, convulsions and salivation.<sup>18</sup> Like many plant alkaloids, the dendrobatid alkaloids clearly have a defensive role. This conclusion is supported by the following observations: (1) the alkaloids are released as a milky (and sometimes odoriferous) secretion by the frogs upon being attacked and following any damage to the skin; (2) snakes, birds and mammals usually release the grabbed frog immediately with evident distaste. Humans also find frog secretions distasteful. Indeed, this is one of the less elegant bioassays used to determine the presence of alkaloids in skin secretions; (3) most of the toxic *dendrobates* are brightly coloured as a warning to predators, non-alkaloid-producing *dendrobates* of the genus *Colostethus* are inconspicuously coloured (usually brown) to avoid detection. The skin secretions have also been suggested to have anti-bacterial and anti-fungal

properties these being useful functions in the dampness of a tropical rain forest.

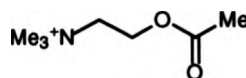


**Scheme 5.** Biosynthetic relationships of dendrobatid alkaloids and precursors

## 2.2.3 Biological activity.

### 2.2.3.1 Cholinergic receptors.

Acetylcholine (Ach) [107] is one of the major neurotransmitters both in the central (CNS) and peripheral nervous systems. Ach is involved as a neurotransmitter in many tissues such as motor nerves, skeletal muscle and certain synapses in the CNS. When an action potential arrives at the cholinergic nerve terminal, Ach is released, crosses the synapse, binds with specialised cell surface receptors known as cholinergic receptors and triggers a response.



[107]

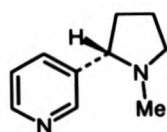
### 2.2.3.2 Subclassification; Nicotinic and Muscarinic receptors.

The terms 'nicotinic' and 'muscarinic' were introduced in 1914 by Sir Henry Dale<sup>44</sup> to describe the effects of Ach that were mimicked by either nicotine [59] (from tobacco, *Nicotiana tabacum*) or muscarine (from the toadstool, *Amanita muscaria*). Some of the better known agonists (stimulators) and antagonists (blockers) are shown in (Figure 4). The two quaternary salts DMPP [108] and PTMA [109] are nicotinic agonists, an antagonist being (+)-tubocurine [110]. Muscarine [111] and arecoline [112] are agonists of the muscarinic receptors, two antagonists being atropine [52] and 3-quinuclidinyl-benzilate [113].

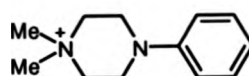
# Ach Receptor

## Nicotinic

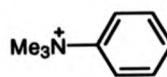
### Agonist



[59]

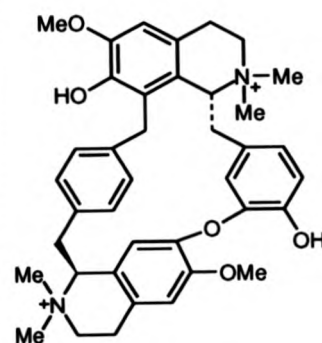


[108]



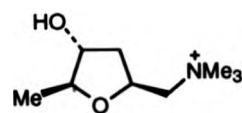
[109]

### Antagonist

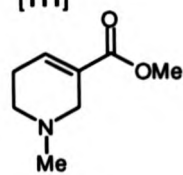


[110]

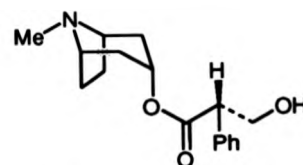
## Muscarinic



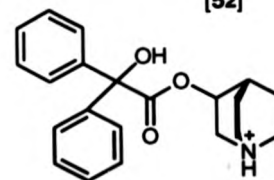
[111]



[112]



[52]



[113]

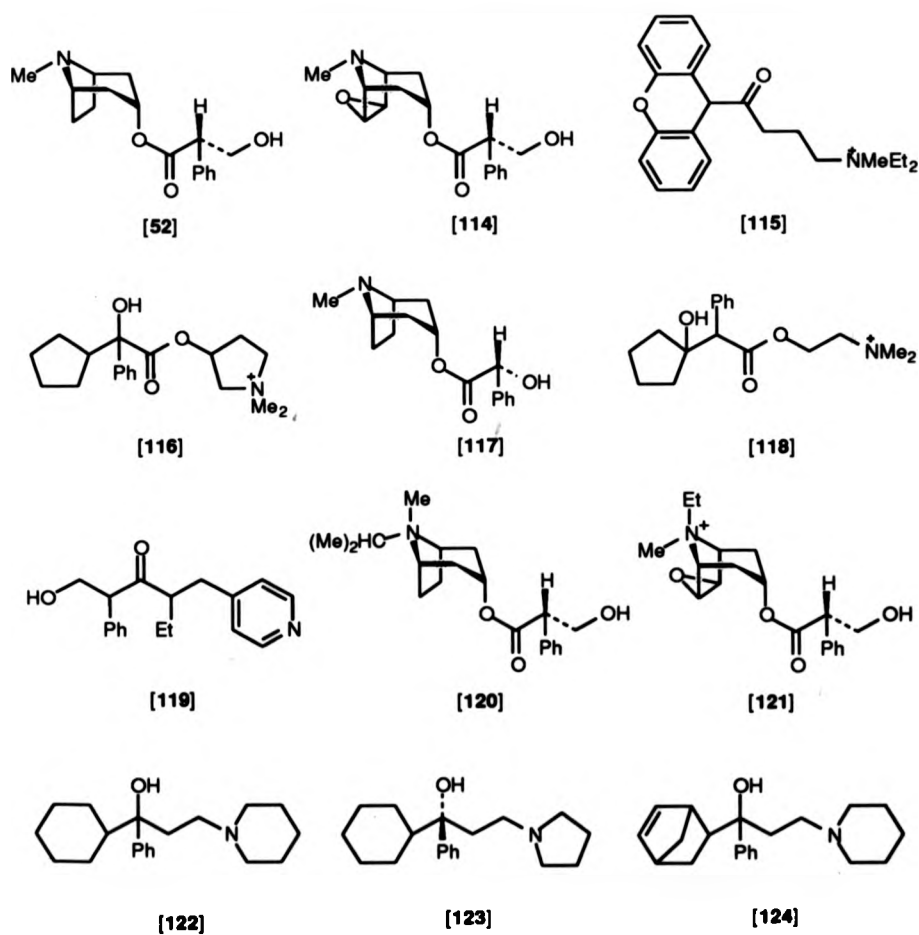
Figure 4. Nicotinic and Muscarinic agonists and antagonists.

### 2.2.3.3 Clinical use.

The indolizidines and perhydrobenzoindolizidines (gephyrotoxins) are competitive muscarinic antagonists, that is they block the action of Ach at muscarinic sites.<sup>45</sup> There are two types of muscarinic receptor sub-types denoted M<sub>1</sub> and M<sub>2</sub>. M<sub>1</sub> receptors are primarily involved in higher brain functions, *viz.* learning and memory, as well as in locomotor and behavioral effects. Indeed, impaired central neurotransmission of cholinergic type is often found in Alzheimer's disease. M<sub>2</sub> type receptors are thought to be primarily involved in the regulation of vegetative, sensory and motor functions. There is little or no published data on whether the indolizidines are M<sub>1</sub> or M<sub>2</sub> preferential. Figure 5 shows some clinically useful muscarinic antagonists. In clinically used doses, atropine [52] and scopolamine [114] induce drowsiness and mild sedation, in larger doses, hallucination and delirium, and in still larger doses death due to paralysis.

These are broad range effects. It is hoped that with the increasing number of synthetic and semi-synthetic antimuscarinic drugs available, soon organ and tissue selective drugs will be developed. The quaternary ammonium compounds methantheline [115] and glucophyrrolate [116], due to their charge, cross the blood-brain barrier to such a small extent that virtually no CNS effects are seen. Homoatropine [117], cyclopentolate [118] and tropicamide [119] are used in ophthalmology to cause dilation of the pupil. Atropine [52] and scopolamine [114] were used in the form of belladonna extract for the same purpose in Victorian times, to dilate the pupils and give the desired and desirable (in those times) wide eyed innocent look to young ladies. Iratropium [120] and oxitropium [121] are administered in aerosol form to cause bronchial dilation. Trihexylphenidyl [122], procyclidine [123] and biperiden [124] cross the blood-brain barrier with ease, acting centrally to

reduce Parkinson-like symptoms by restoring the balance of cholinergic and dopaminergic neurotransmission though undesirable side effects such as hallucinations may limit the usage.



**Figure 5.** Clinically used Muscarinic antagonists

#### 2.2.3.4 Structural requirements of muscarinic antagonists.

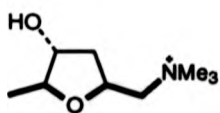
Several structural elements seem to be common to all these agents:<sup>44</sup>

- a 'cationic head' group;
- some sterically 'blocking' moieties (*e.g.* an alicyclic/aromatic system);
- some structural element (*e.g.* ester group) linking the above two;
- anchoring groups (*e.g.* OH) that contribute to the total binding.

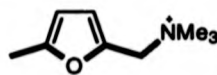
As might be expected, agonists and antagonists are often structurally similar. Both need to bind to the receptor in question, one eliciting a response, the other no response. The requirements for an agonist seem to be:

- a 'cationic head' group;
- either lipophilic (alkyl groups) or electron-rich (*e.g.* ester group, triple bond) moieties.

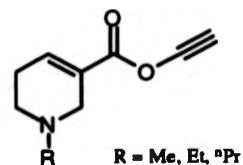
As a rule of thumb, Ing's five atom rule<sup>46</sup> holds true for many muscarinic agonists. This states that there should be a five-atom chain attached at one end to a quaternary nitrogen, the other terminating in a methyl group [111], [125]. Studies on the muscarinic agonist arecaidine proparagyl ester analogues [126] showed that unsaturation in the side chain increased the potency with respect to fully saturated esters.<sup>44</sup>



[111]



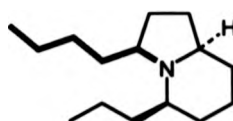
[125]



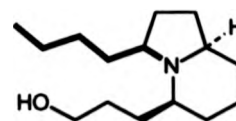
[126]  
R = Me, Et, <sup>n</sup>Pr



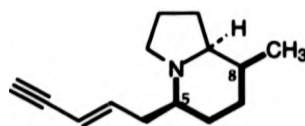
Dendrobatid indolizidines fulfil many of the criteria for both agonists and antagonists. For example, the 3,5-disubstituted 223AB [70] and 239AB [71], along with all the known 5,8-disubstituted indolizidines, *e.g.* 203A [73] and 235B [127] fulfil Ing's five atom rule. They have the required charged head group (at physiological pH), contain alicyclic 'blocking groups' and have 'anchoring groups' such as hydroxyl groups and unsaturation in the side chains. So, some agonist/antagonist behaviour could be predicted from the above. Experiments on mixtures of the indolizidines would seem to agree with this, but clearly tests would have to be carried out on pure compounds to gather more detail on the agonist/antagonist structural requirements. The indolizidines would seem ideal, in the range produced by dendrobatid frogs and structural analogues, to probe the muscarinic receptors, gather structural information about the receptors and perhaps be of clinical use.



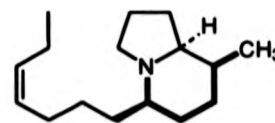
[70]



[71]



[73]

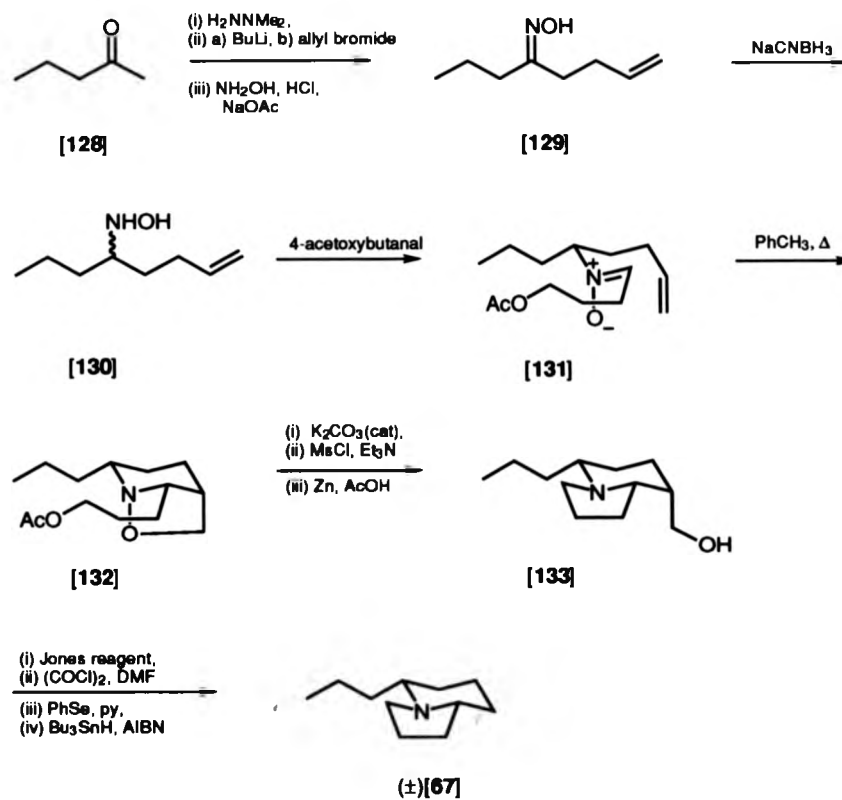


[127]

## 2.3 Chemical syntheses.

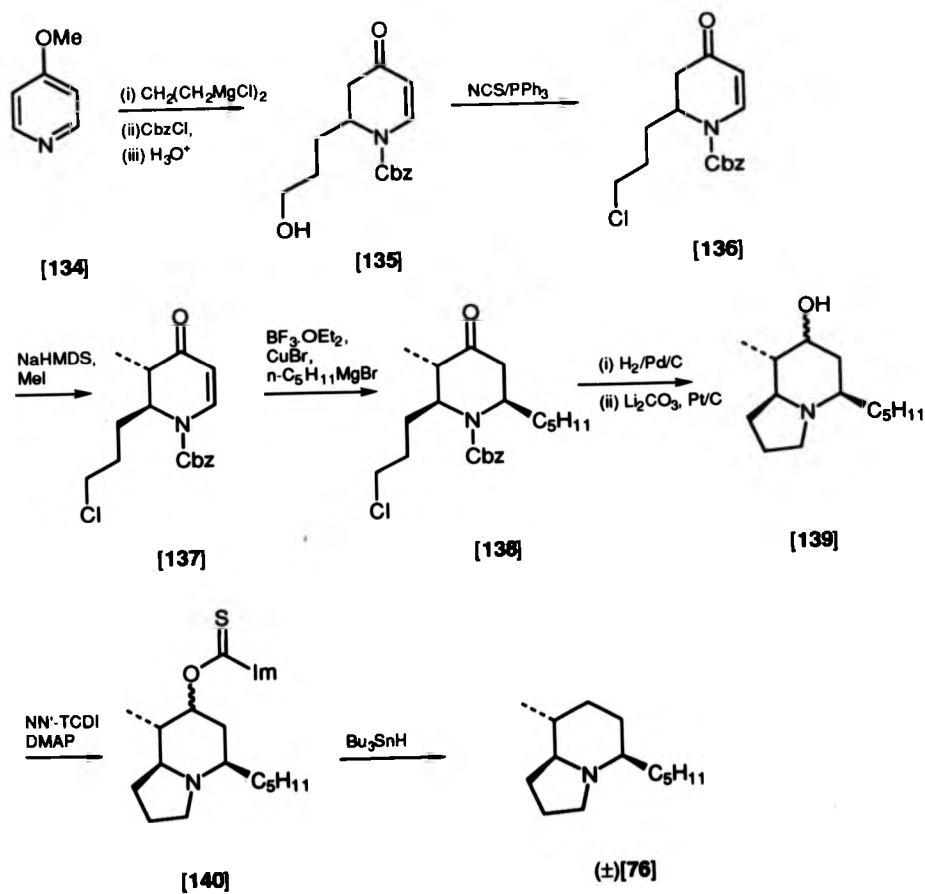
### 2.3.1 Racemic syntheses.

The syntheses of racemic ( $\pm$ )-indolizidines 167B [67] (Scheme 6), 205A [74] and 207A [75] were first reported by Holmes *et al.*<sup>47,48</sup> Intramolecular cycloaddition of the (Z)-N-alkenyl nitron [131] prepared in seven steps from 2-pentanone [128], gave the isoxazolidine [132]. Alkaline hydrolysis of the acetate group, mesylation, accompanied by spontaneous cyclization and reductive N-O bond cleavage with Zn/AcOH furnished the 5,8-disubstituted indolizidine [133]. Removal of the C-8 hydroxy-methylene substituent was achieved by oxidation to the carboxylic acid, then decarboxylation *via* the seleno-ester yielding ( $\pm$ )-167B [67]. The three new stereogenic centres in [133], the 5, 8 and 9 positions were controlled by the single stereogenic centre  $\alpha$  to the nitrogen in nitron [131]. This method has been extended to ( $\pm$ )-235B [127] and to the asymmetric synthesis of (-)-209AB [76], discussed in section 2.3.4.



Scheme 6

Commins *et al.*<sup>49</sup> through investigations in the chemistry of *N*-acyldihydropyridones, prepared (±)-209B [76] in seven steps from 4-methoxypyridine [134] (Scheme 7). Dihydropyridone [137] was prepared from 4-methoxypyridine [134] and Grignard reagent (i), after protection of the amine with benzylchloroformate. Chlorination with *N*-chlorosuccinimide/triphenyl phosphine, followed by stereoselective methylation and cuprate addition gave [138]. Hydrogenation with palladium to remove the Cbz group, platinum catalyzed hydrogenation to reduce the carbonyl and Barton deoxygenation yielded (±) 209B [76].



Scheme 7

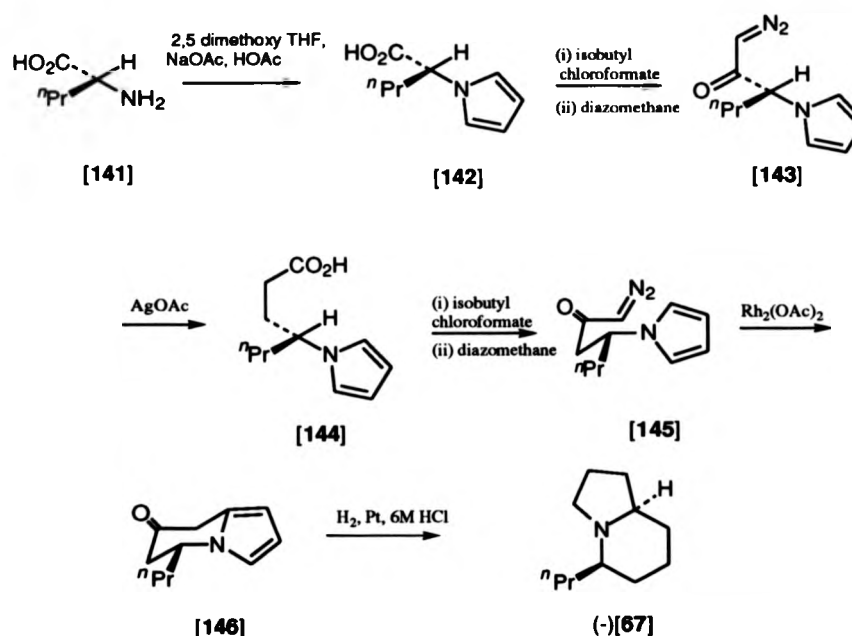
### 2.3.2 Asymmetric syntheses

A symmetric synthesis would, by definition, produce a statistical mixture of enantiomers and diastereomers *i.e.* a racemate. An asymmetric synthesis, to be anything other than a mechanistic curiosity, needs to produce one of the possible enantiomers in greater than 90% *e.e.* The new chiral centres need to be supplied by the substrate, the reagent or a catalyst present. Aitken and Kilenyi<sup>50</sup> propose the following classification of asymmetric syntheses.

### 2.3.3. First generation or substrate controlled methods.

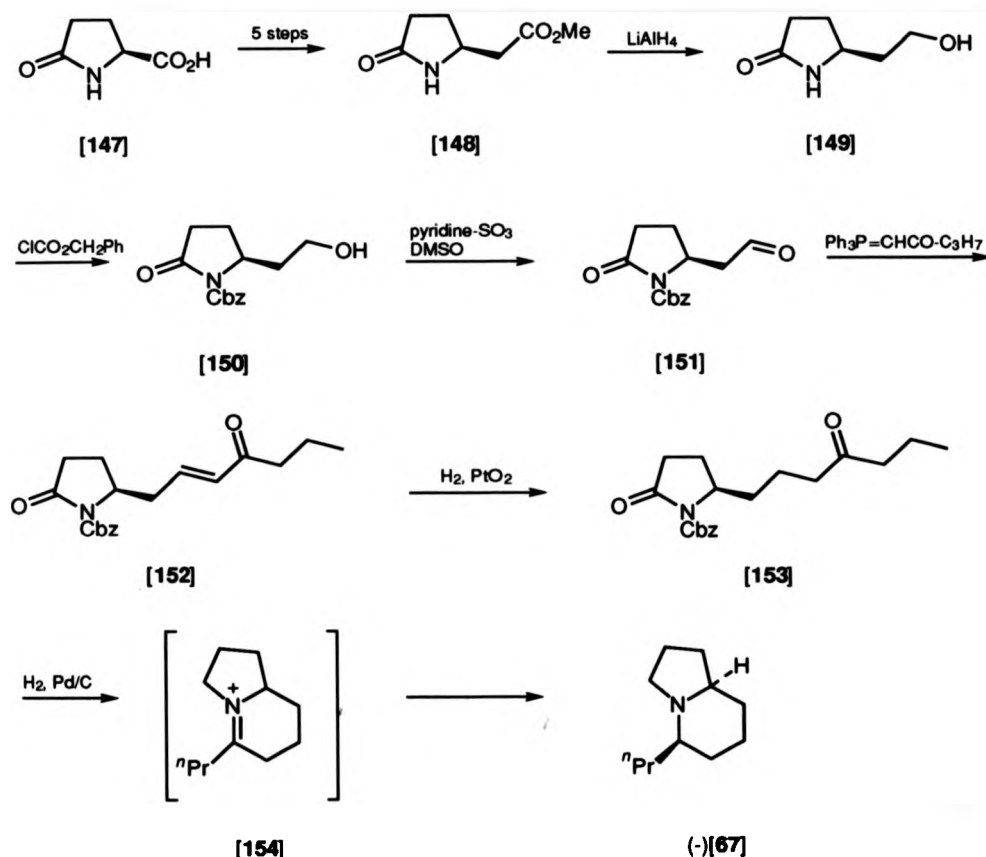
The ultimate source of chirality is nature. Amino acids (with few exceptions) are of the *L* variety whilst natural carbohydrates are of the *D* variety. This provides a vast 'chiral pool' of readily available starting materials. Several syntheses of indolizidines have made use of chiral natural products to introduce stereochemistry.

(-)-Indolizidine 167B [67] has been prepared using D-norvaline [141] as the chiral building block (Scheme 8).<sup>51</sup> The pyrrole [142] formed from the condensation of 2,5-dimethoxytetrahydrofuran and D-norvaline [141] was converted to the  $\alpha$ -diazo ketone [143]. Arndt-Eistert homologation (to introduce the extra methylene group) gave the second  $\alpha$ -diazoketone [145] which, upon rhodium acetate-mediated cyclization, gave the  $\beta$ -keto pyrrole [146]. Hydrogenation with Adams catalyst under acidic conditions yielded the desired (-)-167B [67].



Scheme 8

Using the rigid amino acid equivalent (S)-pyroglutamic acid [147] Fleurant *et al*<sup>52</sup> also synthesised (-)-167B [67] (Scheme 9). (S)-pyroglutamic acid [147] was converted to the methyl ester [148] in five steps by one-carbon homologation using nitrile chemistry. Reduction and N-protection yielded the alcohol [150] which was subjected to Swern oxidation and subsequent Wittig elongation to give the  $\alpha,\beta$ -unsaturated ketone [152]. Hydrogenation over platinum oxide gave the ketone [153] which was further hydrogenated over palladium. This accomplished the reduction of the amide to an amine functionality and N-deprotection, with subsequent cyclization and reduction of the iminium [154] thus produced to the indolizidine (-)-167B [67].

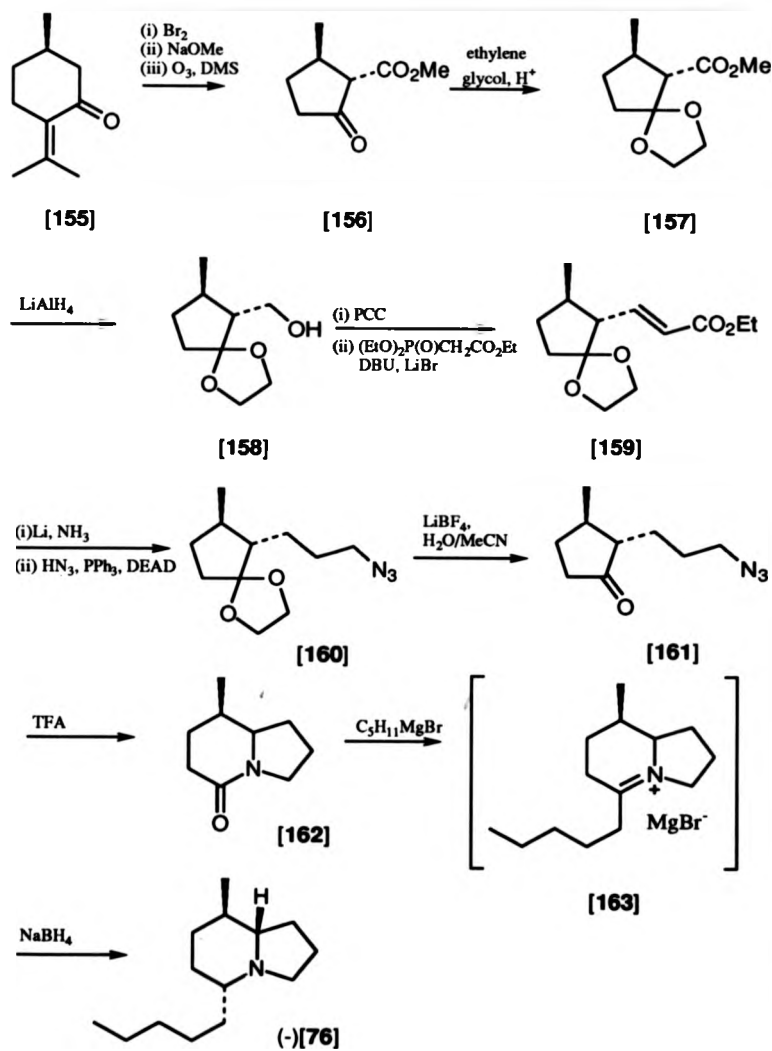


Scheme 9

Amino acids are not the only natural products that have been used for the synthesis of 5-mono- and 5,8-di-substituted indolizidines. Aubé *et al* began their synthesis of (-)-209B [76] with pulegone [155] (Scheme 10).<sup>53</sup> Pulegone [155] contains the 8-methyl group required for (-)-indolizidine 209B [76]. Ring contraction of pulegone [155] *via* Favorskii rearrangement, and conversion to the  $\beta$ -keto ester [156] was first reported by Marx and Norman.<sup>54</sup> Here the *trans* isomer is produced predominantly (*cis:trans*, *ca* 1:13); the *cis* isomer ultimately removed by fractional crystallisation. Protection of the keto-group of [156] yielded the acetal [157] which was reduced to the primary alcohol [158]. The alcohol [158] was then oxidised to the aldehyde and submitted to Horner-

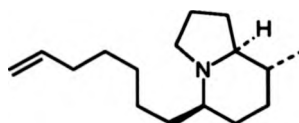
Emmons extension resulting in the  $\alpha,\beta$ -unsaturated ester [159]. Dissolving metal conditions reduced the ester and the alkene functionalities and Mitsunobu conditions were used to produce the azide [160]. Ketal removal and an intramolecular Schmidt reaction yielded, after recrystallisation, enantiomerically pure [162]. Attack by *n*-pentyl magnesium bromide and stereospecific reduction of the iminium ion intermediate [163] yielded (-)-209B [76].





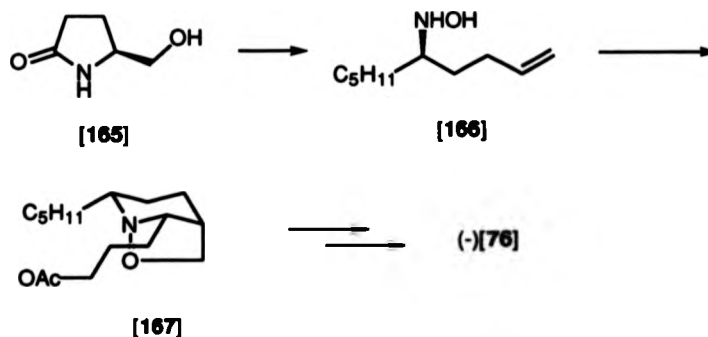
Scheme 10

As mentioned before, Holmes *et al* have used the nitron methodology shown in Scheme 6 to synthesise the indolizidines ( $\pm$ )167B[67], ( $\pm$ )205A[74], ( $\pm$ )207A[75], ( $\pm$ )235B[127] and ( $\pm$ )235B[164]. This methodology has also been used to produce enantiomerically pure indolizidines.



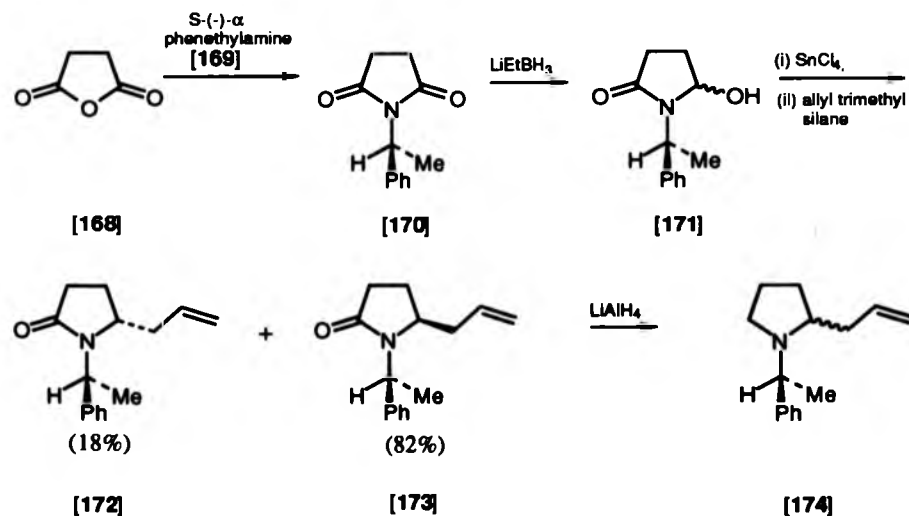
[164]

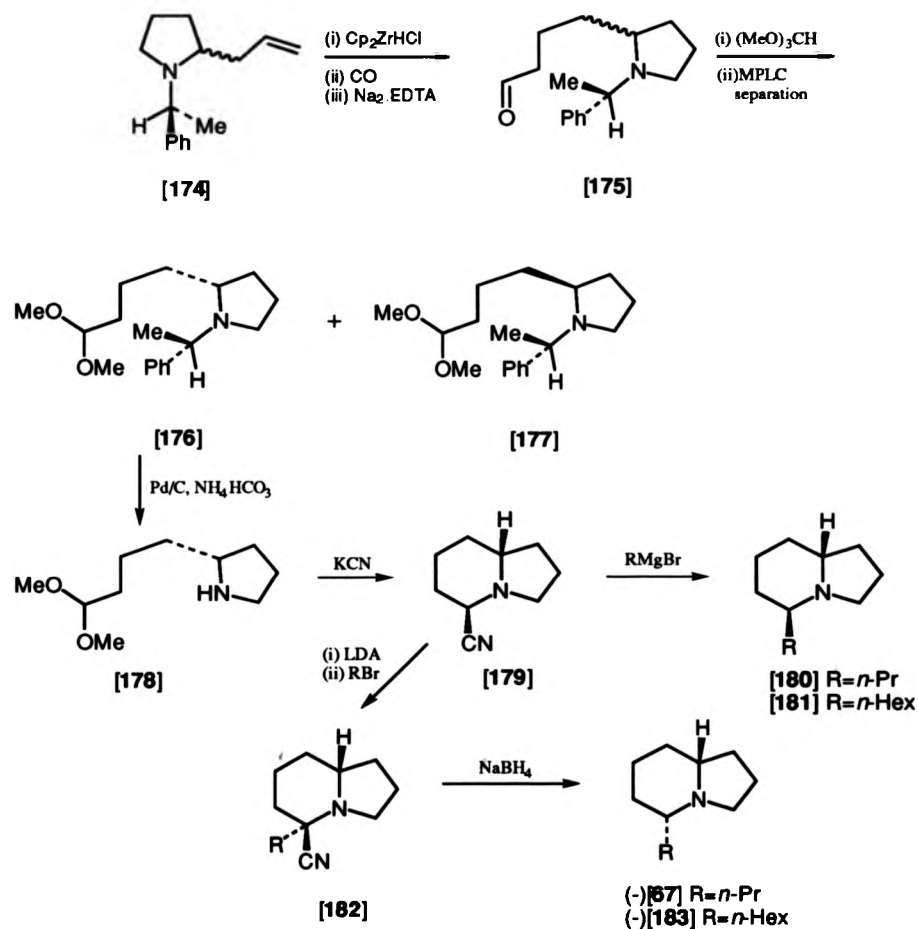
In the Holmes synthesis of (-)-indolizidine (-)-209B [76],<sup>48</sup> Holmes and co-workers used the nitron [131] as the reagent. The synthesis of (-)-209B (-)[76] started with the preparation of the enantiomerically pure N-alkenylhydroxylamine [166] (derived from the chiral amino alcohol [165]) (Scheme 11). This was achieved in 12 steps, using a chain extension sequence. The nitron [167] was produced and converted into (-)-209B [76] using a previously reported sequence.<sup>47</sup>



Scheme 11

Polaniaszek and Belmont<sup>55</sup> undertook a synthesis of indolizidines (-)-167B [67], and (-)-209D [183] and their diastereomers from (S)-(-)- $\alpha$ -phenethylamine [169] (Scheme 12). The aldehydes, [175] produced in five steps from succinic anhydride [168] and (S)-(-)- $\alpha$ -phenethylamine [169] were converted to the corresponding dimethyl acetals [176] and [177] which were separable by MPLC. The major isomer [176] was hydrogenated using Pd/C to deprotect the amine, then hydrolysed in the presence of hydrogen cyanide to remove the acetal with concomitant cyclization and trapping of the iminium ion with cyanide. This produced the amino nitrile [179] as a single isomer. This amino nitrile thus served as a late-stage common intermediate which upon treatment with an appropriate Grignard reagent, furnished [180] and [181]. Treatment of [179] with base and an alkyl bromide gave [182] followed by sodium borohydride reduction produced the epimers (-)-167B [67] and (-)-209D [183].

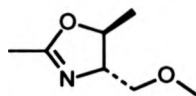




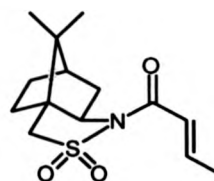
Scheme 12

### 2.3.4 Second-generation or auxiliary-controlled methods

In the auxiliary-controlled approach, control is again achieved intramolecularly by a chiral group in the substrate. The difference is that the 'chiral auxiliary' directing the control is now deliberately attached to an achiral substrate and removed later in the synthesis once the stereocontrol has been achieved. Examples of this are the Meyers oxazolidine [184] and Oppolzer's camphor sultams [185].<sup>50</sup>



[184]



[185]

The asymmetric synthesis of (+)-209B [76] was approached by Gnecco *et al*<sup>56</sup> by utilising the innate chirality of (*R*)-(-)-phenyl glycinol [187] in the pyridinium salt [188], (Scheme 13) gained from the Zinck's salt [186]. Reduction with sodium thiosulphate yielded the 1,4-dihydropyridine [189] which cyclised to the two oxazolidines [190a] and [190b] when filtered through alumina. These, being masked iminium ions, upon treatment with *n*-pentyl magnesium bromide cleaved the heterocycle replacing oxygen with a pentyl group. Spontaneous cyclization again occurred on filtering through alumina to yield the two new oxazolidines [191a] and [191b]. These were again selectively cleaved with a Grignard reagent producing a mixture of the piperidines [192], [193] and [194] only, in the ratio, 5:3:1. Separation on silica gel and subsequent hydrogenation yielded (+)-209B [76], (-)-209B [76] and the diastereomer [195].



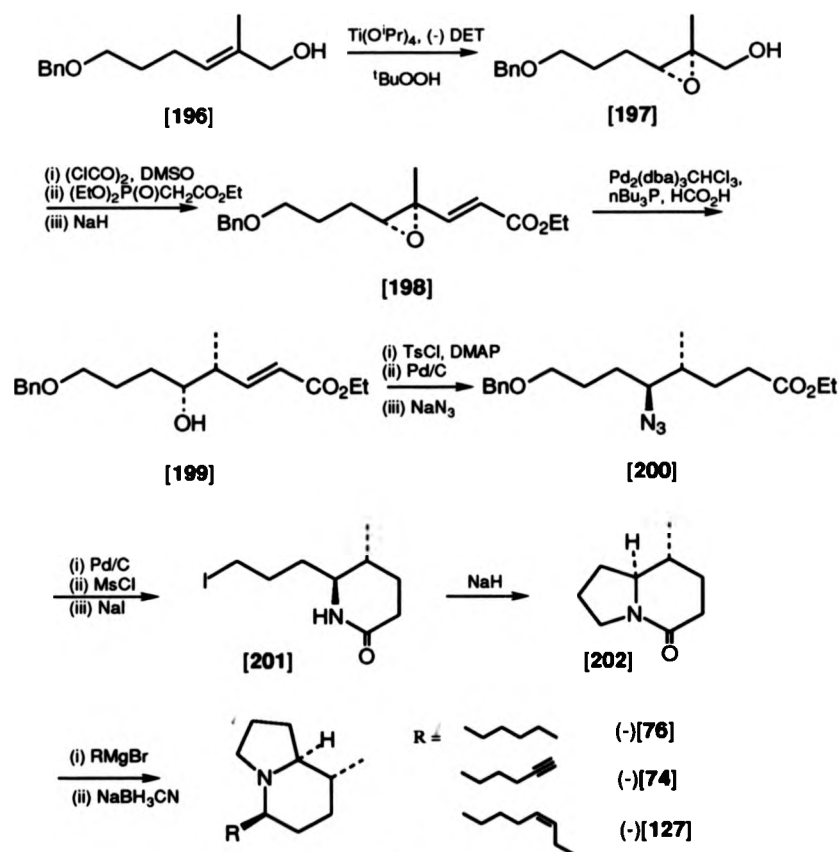
### 2.3.5 Third-generation or reagent controlled methods.

In the third-generation methods, in contrast to first and second, the stereocontrol is intermolecular, the reagent, not the starting material, being homochiral. In the area of the 5-mono or 5,8-disubstituted indolizidine synthesis there is no clear example to illustrate this method of asymmetric induction.

### 2.3.6 Fourth-generation or catalyst-controlled methods.

A chiral catalyst removes the need for a stoichiometric amount of an enantiomerically pure compound. This is an attractive refinement to the methods detailed above. At the moment there are limited numbers of synthetic chiral catalysts, though there are a number of synthetically useful enzymes.

An example of the use of chiral catalysis is the exploitation of the Sharpless epoxidation by Satake *et al*<sup>57</sup> in the synthesis of (-)-209B [76], (-)-235B [127] and (-)-205A [74] (Scheme 14). The alkenyloxirane [198] was prepared from the allylic alcohol [196] by sequential Sharpless epoxidation, Swern oxidation and Emmons-Horner chain elongation, the Sharpless epoxidation introducing the two new chiral centres in the form of the epoxide [197]. Hydrogenolysis of [198] with a palladium catalyst in formic acid gave the homochiral alcohol [199]. This was converted to the iodide [201] in seven steps *via* the azide [200]. Cyclization gave the bicyclic ketone [202] as a late stage common intermediate for the synthesis of the indolizidines. Treatment with the appropriate Grignard reagent and sodium cyanoborohydride reduction yielded (-)-209B [76], (-)-235B [127] and (-)-205A [74].

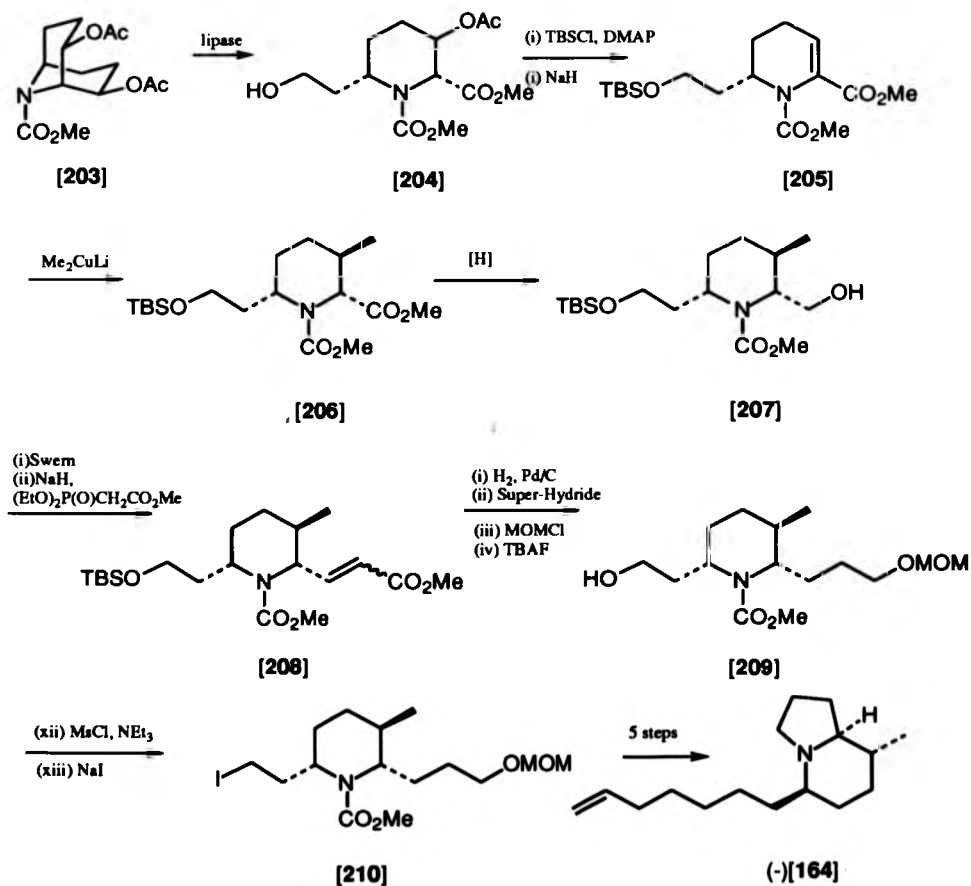


Scheme 14

Momose used a lipase to prepare the homochiral piperidine **[204]** from the diacetate **[203]**.<sup>58</sup> Protection and reduction of **[204]** gave the  $\alpha,\beta$ -unsaturated ester **[205]** which underwent stereospecific methyl addition to produce what would become the 8-methyl group of the indolizidines (-)-207A **[75]**, (-)-209B **[76]** and (-)-235B' **[164]**. Reduction gave the alcohol **[207]** which, under Swern oxidation conditions, followed by Wittig-Horner chain elongation of the resulting aldehyde, gave a 4:1 mixture (*E:Z*) of the  $\alpha,\beta$ -unsaturated ester **[208]**. Catalytic hydrogenation followed by Super-hydride reduction of the intermediate aldehyde and protection of the resultant alcohol furnished **[209]**, which was then converted to the iodide **[210]**. Copper iodide mediated



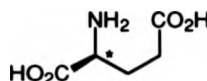
Grignard cross-coupling followed by deprotection of both the alcohol and the amine led to intra-molecular cyclodehydration *via* the alkoxy phosphonium salt when exposed to  $\text{PPh}_3/\text{CBr}_4$  and triethylamine (first reported by Machinaga<sup>59</sup>). This forms (-)-235B' [164] (Scheme 15). The syntheses of (-)-207A [75] and (-)-209B [76] were also reported by Momose.<sup>58</sup>



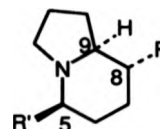
Scheme 15

## 2.4 Summary of New Methodology.

(L)-Glutamic acid [211] and the 5-mono and 5,8-disubstituted indolizidines [212] and [213] have several structural similarities.



[211]

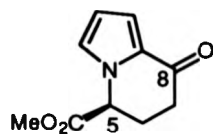


[212] R = H,  
R' = alkyl, alkenyl

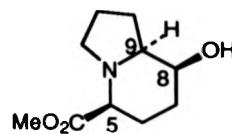
[213] R = Me  
R' = alkyl, alkenyl

(L)-Glutamic acid [211] has an amine which is  $\alpha$  to the chiral centre (\*) of the same absolute configuration as the parent indolizidines [212], [213]. The  $\delta$ -acid function is suitably positioned to facilitate formation of C(8)-C(9) bond of the indolizidines and allow installation of the 8-substituent. In view of this, L-glutamic acid was used as a starting point for the construction of the indolizidine skeleton.

The missing 4-carbon fragment could be supplied by pyrrole formation which would permit intramolecular Friedel-Crafts acylation to form the important C(8)-C(9) bond. Reduction of the  $\alpha$ -ketopyrrole [214] so produced should furnish the indolizidine alcohol ester [215]. The C(5) ester group was expected to direct the hydrogenation to the *endo* face of the molecule thus giving *cis* addition of H<sub>2</sub> and setting up the correct relative stereochemistry as in [215].



[214]



[215]

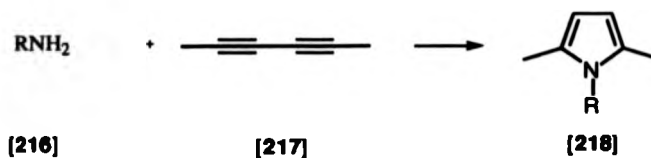
Conversion of the 8(*S*)-hydroxyl group to an appropriate leaving group and an  $S_N2$  methylation would provide the 8(*R*)-methyl group of the 5,8-disubstituted indolizidines. Homologation of the 5-ester group should lead to the desired indolizidine side-chains.

## 2.5 Pyrrole formation

There are several methods by which pyrroles may be produced,

### 2.5.1 Reaction with conjugated diynes.

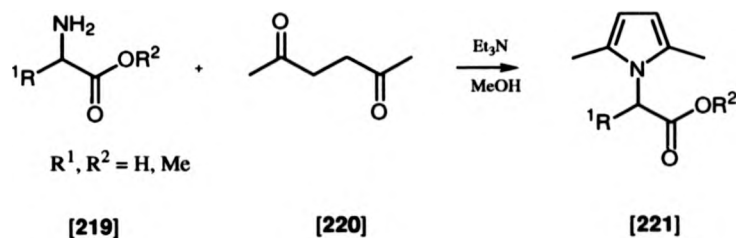
Ammonia and primary amines [216] (both aliphatic and aromatic) add to conjugated diynes [217] to produce pyrroles [218] (Scheme 16).<sup>60</sup>



Scheme 16

### 2.5.2 Reaction with $\gamma$ -dicarbonyls.

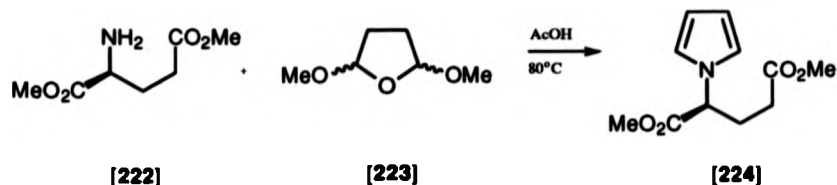
Pyrroles are readily synthesised from primary amines and  $\gamma$ -dicarbonyl compounds. Paal and Knorr reported that *N*-substituted 2,5-dimethyl pyrroles [221] were prepared from the corresponding amine [219] and acetylacetone [220].<sup>61,62</sup> This is now known as the Paal-Knorr pyrrole synthesis (Scheme 17).



Scheme 17

### 2.5.3 Reaction with 2,5 disubstituted tetrahydrofurans.

Simple *N*-substituted pyrroles [224] can be derived from primary amines *e.g.* [222] and 2,5-dimethoxytetrahydrofuran [223]<sup>63</sup> or 2,5-dichlorotetrahydrofuran. The use of 2,5-dimethoxytetrahydrofuran [223] is preferable (Scheme 18), as it is more stable.



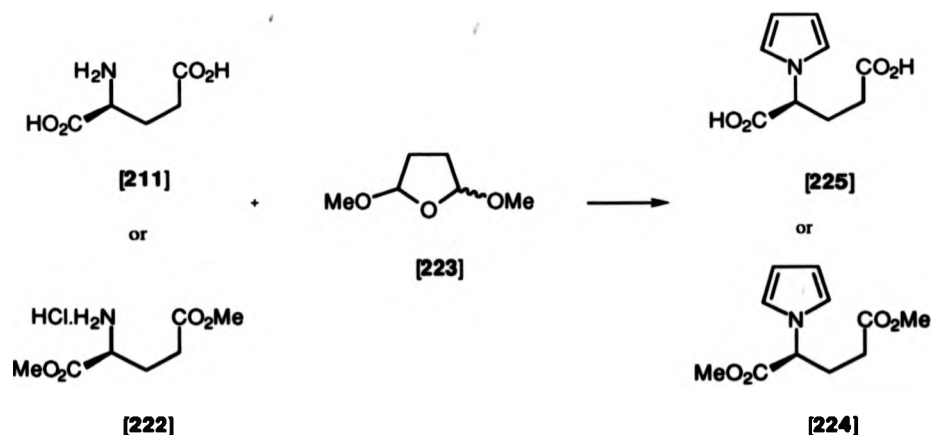
Scheme 18

The last of these three routes was considered preferable, since Kashima and co-workers<sup>64</sup> had already synthesised the pyrrole dimethyl ester [224] and

reported that the *N*-pyrrole formation proceeded with no significant racemization. Indeed, they found that when phenylalanine was the starting material, retention of the optical activity of the amino acid from pyrrole formation and cleavage was in excess of 99%. So, we were confident in the configurational integrity installed by the choice of *L*-glutamic acid as the chiral starting material.

## 2.5.4 Results and Discussion.

The synthesis of [224] was first attempted using the conditions described by Kashima. However, the modified Clauson-Kaas procedure described by Jefford<sup>65</sup> proved more successful. Hence 2,5-dimethoxy tetrahydrofuran [223] was added to the hydrochloride of *L*-glutamic acid dimethyl ester [224] in acetic acid with an excess of sodium acetate (Scheme 19).

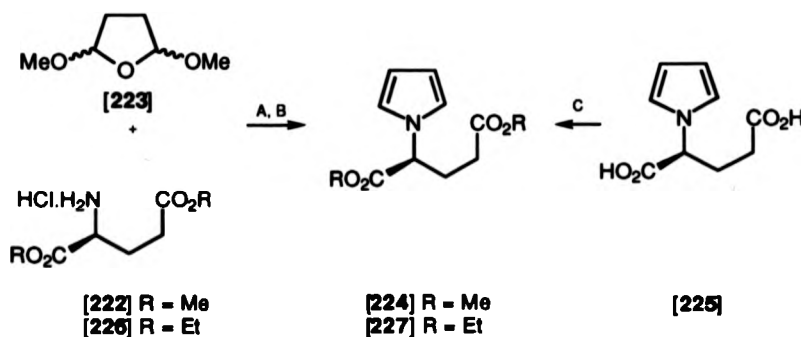


Scheme 19

The previously unreported *N*-pyrrole diacid [225] was also prepared from *L*-glutamic acid [211] using the conditions described above, as racemization in the form of the diacid would be less likely. Characterisation of this was

undertaken using  $^1\text{H}$  and  $^{13}\text{C}$  NMR as CHN and accurate mass measurements were problematic, possibly due to decomposition. Problems were also encountered with the CHN analysis of other samples that were liquids due to calibration and general difficulties of handling oils. Diacid [225] was converted to either the dimethyl or diethyl ester by refluxing in the appropriate alcohol in the presence of the acidic ion-exchange resin Amberlyst 15.

The diethyl ester [227] was recently reported by Jefford<sup>65</sup> to be prepared in good yield by the alternative conditions of water at 80°C for 30 minutes. Investigations were undertaken on the preparation of the diester analogues under both the modified Clauson-Kass and Jefford's procedures. This showed that some epimerization had occurred during the reaction in glacial acetic acid (Scheme 20), Table 1. This was confirmed by a later paper<sup>66</sup> by Jefford who investigated the preparation of both the diester analogues and found their method to be superior in avoiding racemization.



Scheme 20

| Entry | Educt | Procedure <sup>@</sup> | Product | Yield, % | [ $\alpha$ ] <sub>D</sub> <sup>*</sup> |
|-------|-------|------------------------|---------|----------|--|
| 1     | 222   | A                      | 224     | 70       | -28.6                                  |
| 2     | 222   | B                      | 224     | 23       | -33.5                                  |
| 3     | 226   | A                      | 227     | 83       | -16.6                                  |
| 4     | 226   | B                      | 227     | 70       | -22.1                                  |
| 5     | 225   | C                      | 224     | 44       | -29.9                                  |
| 6     | 225   | C                      | 227     | 52       | -17.0                                  |

<sup>@</sup> Procedures; A - Acetic acid, reflux, 30 mins; B - Water, 80 deg, 30 mins; C - Amberlyst 15, ROH, reflux 2 days.

<sup>\*</sup>At this time it should be mentioned that [ $\alpha$ ]<sub>D</sub> values can only be strictly compared if the concentration of the sample and the other variables are in accordance.

**Table 1**

This data shows that epimerization is indeed a problem even in the preparation of the diacid [225], despite the literature precedent. So, preparation of the pyrrole diethyl ester under Jefford's non-epimerizing conditions was undertaken a) to avoid epimerization, b) as the yields for the ethyl ester are higher than those of the dimethyl ester under these conditions and c) L-glutamic acid diethyl ester hydrochloride is cheaper than its dimethyl ester hydrochloride and purification by distillation of the ethyl ester pyrrole [227] gave better yields than the methyl ester [224].

## 2.6 Cyclization.

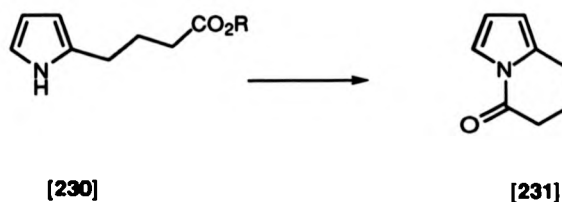
### 2.6.1 Introduction.

There are two possible entries into the bicyclic pyrrole system, the first being the formation of the *N*-substituted pyrrole [228] with the subsequent cyclization at the pyrrole  $\alpha$ -position yielding [229] (Scheme 21). The second

was the formation of an  $\alpha$ -substituted pyrrole [230] followed by subsequent *N*-acylation to yield [231] (Scheme 22).



Scheme 21



Scheme 22

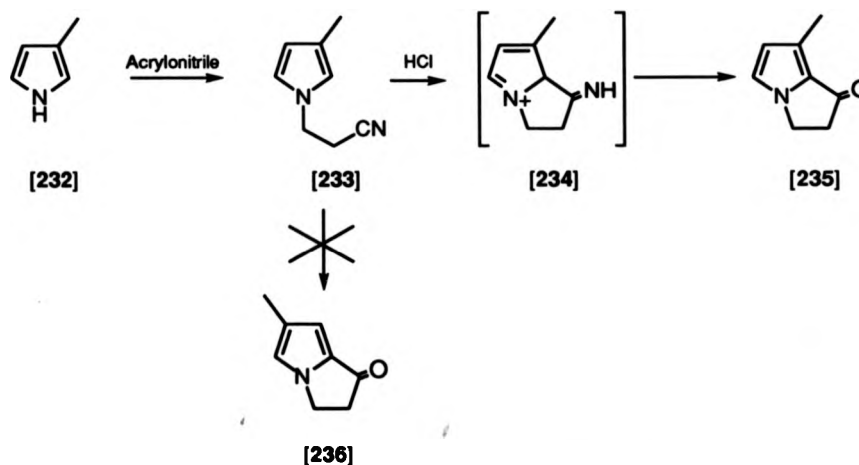
The first of the two possibilities allowed a first-generation method of attaining the C(5) chiral centre of the parent indolizidines. The second route did not lend itself to installation of chirality in this way. Also, the second of the two methods requires selective prior  $\alpha$ -acylation of pyrrole, which can be problematic and is often accompanied by intermolecular acylation.

## 2.6.2 Methods of C-acylation of pyrroles.

Most of the literature precedents for C-acylation of pyrroles at the  $\alpha$ -position have been carried out on pyrrole itself or simple alkyl or aryl derivatives *e.g.* 1-methyl, 2,4-dimethyl, 2,3,5-trimethyl and 2,3,5-triphenyl pyrrole.<sup>67</sup> Formylation, either at the  $\alpha$ - or the  $\beta$ -position has been extensively



carried out on simple pyrroles using the Gatterman reaction. This involves the addition of anhydrous hydrogen chloride to a solution of the pyrrole [233] and hydrogen cyanide (or zinc cyanide in ether or chloroform.)<sup>68,69,70</sup> The intermediate iminium salt [234] is hydrolysed during the work-up. Meinwald and Meinwald<sup>71</sup> used this method to synthesise [235], the major component of the hairpencil secretion of the butterfly *Lycorea ceres ceres* (Scheme 23).



Scheme 23

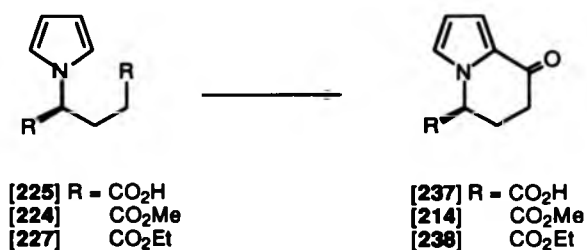
They found that only one of the possible cyclization products [235] was formed. They put forward the explanation that selective activation of C-2 by the 3-methyl group to the intramolecular electrophilic substitution produced [235] rather than [236].

The Vilsmeier-Haack synthesis, the use of benzoyl chloride in conjunction with DMF,<sup>72</sup> fails to produce  $\alpha$ -formylated pyrroles<sup>73</sup> if the molecule contains electron-withdrawing groups. Ethyl orthoformate in the presence of a Lewis acid catalyst has been used to formylate phenol and azulene.<sup>74,75</sup> Clezy *et al*<sup>76</sup> found formylation was easily effected by reaction of ethyl orthoformate in the presence of trifluoroacetic acid. They used this method to produce a wide

variety of  $\alpha$ - and  $\beta$ -formyl pyrroles, though they found that this was most effective when the desired product contained the  $\beta$ -formyl group.

It can be concluded from this that the most promising method of  $\alpha$ -C-acylation would be the intramolecular reaction of either the diacid [225] or the diesters [224] and [227]. This would avoid the problems of (i) C- vs N-acylation (selection between the N-acyl and C-acyl products can be achieved by either addition of a base (*e.g.* triethylamine) to favour the former or the addition of a Lewis acid or a trace of sulfuric acid to ensure C-acylation of the pyrrole<sup>77</sup>) and (ii)  $\alpha$ - vs  $\beta$ -acylation. The length of the methylene tether would favour, both kinetically and thermodynamically, the formation of a 6 vs 7 membered ring.

In summary, the electron-rich pyrrole system coupled with the conformational restraints of an intramolecular reaction was expected to effect facile C-acylation at the  $\alpha$ -position of the pyrrole ring to produce the  $\alpha$ -keto pyrroles [237], [214] and [238] (Scheme 24).

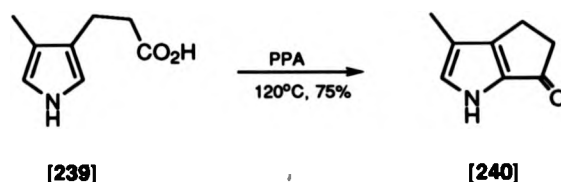


Scheme 24

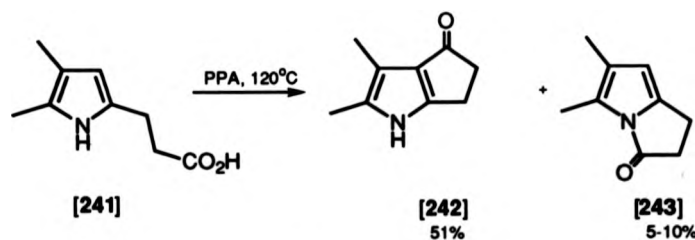
Friedel-Crafts acylation is effective using acyl halides, carboxylic acids, anhydrides, ketones<sup>78</sup> and esters (though alkylation products are often a problem). Reactive pyrroles may be formylated under mild conditions by reaction in the presence of a Lewis acid such as stannous chloride or aluminium chloride,<sup>79</sup> though a loss of selectivity is seen and, due to the

heterogeneous nature of the pyrrole/acid catalyst complex, polymer formation is often a problem. Other Friedel-Crafts catalysts that have been used include magnesium perchlorate,<sup>80,81</sup> stannic chloride,<sup>82</sup>  $\text{FeCl}_3$ ,  $\text{I}_2$ ,  $\text{ZnCl}_2$ , Fe as well as proton acids such as polyphosphoric acid (due to its high potency) and sulfuric acid, though the use of these is usually restricted to where the substrate itself is an acid.

Cyclization of a 3-substituted pyrrole acid [239] was achieved by refluxing with polyphosphoric acid yielding the bicyclic pyrrole [240]. (Scheme 25).<sup>83</sup> Volz and Draese<sup>84</sup> undertook a similar cyclization of the pyrrole [241], yielding a mixture of products [242], [243]. (Scheme 26).



Scheme 25

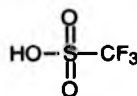


Scheme 26

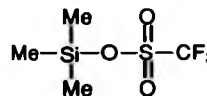
The fact that pyrrole can be acetylated with acetyl trifluoroacetate<sup>85</sup> and that pyrrole acetylation is catalysed by boron trifluoride,<sup>86</sup> as well as the examples above, encouraged us to look at other, newer Friedel-Crafts catalysts to effect the cyclization. Trifluoromethanesulfonic acid [244] (triflic acid) and

derivatives, *e.g.* trimethylsilyltrifluoromethane sulfonate [245] first reported by Haszeldine and Kidd,<sup>87,88</sup> has many advantages over other acid systems:<sup>89</sup>

- It is one of the strongest acids known;
- It is non-oxidizing;
- It doesn't provide fluoride ions;
- It possesses superior thermal stability and resistance to both oxidation and reduction;
- The reaction is homogeneous thus reducing possible polymer formation.



[244]



[245]

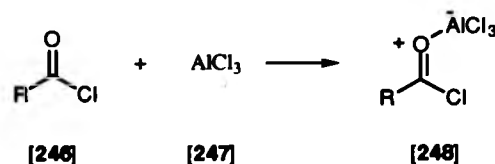
Butler and Kray have shown that triflic acid is superior to aluminium chloride in the acylation of *p*-xylene<sup>90</sup> and in alkylation.<sup>91</sup>

Marsman<sup>92</sup> measured the <sup>29</sup>Si-NMR shifts of a series of trimethylsilyl derivatives (CH<sub>3</sub>)<sub>3</sub>SiX where X ranges from CN, Br, F, Cl to SO<sub>4</sub>, ClO<sub>4</sub>, OSO<sub>2</sub>CF<sub>3</sub>. They estimated pK<sub>a</sub> values of these Lewis acids and showed perchlorate and triflate were stronger acids than the others in the series. A major advantage of the triflate and others of these novel catalysts over SnCl<sub>4</sub> is their lowered Lewis acidity. This dramatically reduces the σ-complex formation with silylated bases, a natural consequence being the higher yields and increased catalytic activity. This is especially well illustrated in nucleoside chemistry.<sup>93</sup> Other advantages over the more "classical" Friedel-Crafts catalysts are mild reaction conditions, easy work-up and, in many cases, improved stereoselectivity.

Initial attempts at Friedel-Crafts acylation were undertaken on the pyrrole dimethyl ester [224] with trimethylsilyltrifluoromethane sulfonate (TMSOTf) as the catalyst. Though no literature precedents could be found for the use of TMSOTf in Friedel-Crafts cyclization of pyrrole systems, this catalyst was investigated with some confidence due to general synthetic utility as a Lewis acid.

### 2.6.3 Results and discussion.

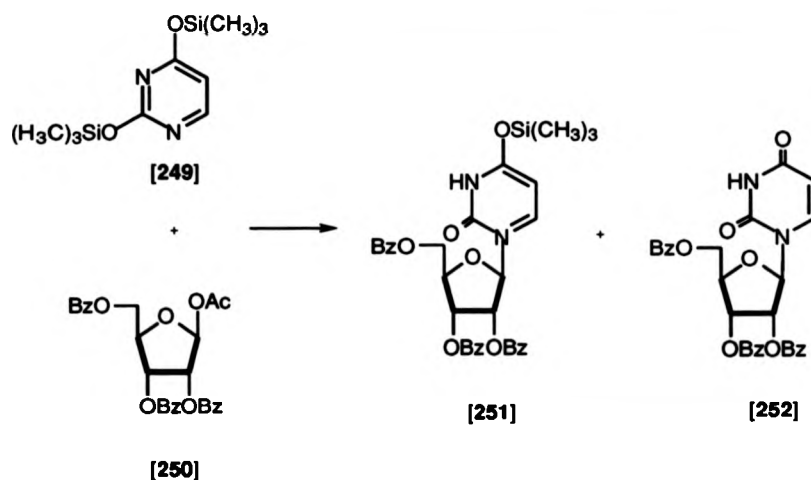
The cyclization was first attempted on the dimethyl derivative as this was the first produced and on the diester rather than the diacid due to the relative ease of purification and general working with the diester. TMSOTf, in theory, could be used in catalytic amounts. In normal Friedel-Crafts acylation (*e.g.*  $\text{AlCl}_3$ ) more than one equivalent is needed as the first equivalent co-ordinates to the carbonyl functionality and remains co-ordinated until work-up destroys this complex [248] (Scheme 27).



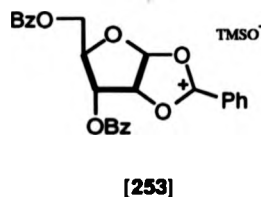
Scheme 27

Therefore, the use of 1.1-1.3 equivalents may be required. Vorbrüggen *et al*<sup>93</sup> has noted the possible catalytic use of TMSOTf and the practical use of more than stoichiometric amounts. They put this down to  $\sigma$ -complex formation between TMSOTf and the silylated base [249] (Scheme 28). This inactivates one equivalent of the TMSOTf, using 1.1-1.3 equivalents dramatically speeds up the reaction and makes the conditions easier to work

with. Alternatively the catalyst was not strong enough to convert the 1-O-acetyl-2,3,5-tri-O-benzoyl- $\beta$ -D-ribofuranose [250] to the reactive electrophilic sugar cation [253].

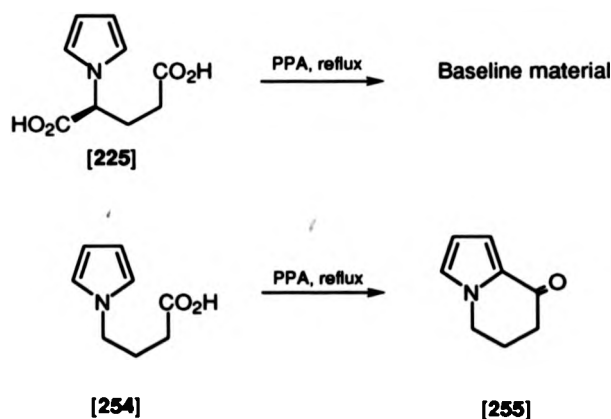


Scheme 28



We also found that the use of TMSOTf did not give practical yields with less than six equivalents of TMSOTf. TMSOTf, being a ~~strong~~ Lewis acid, can accept electron density from the many electron-rich moieties, *e.g.* double bonds, esters and the tertiary amine. So, the pyrrole dimethylester [224] can coordinate five equivalents of TMSOTf and the sixth and further equivalents undertake the reaction. As TMSOTf is relatively expensive, the need for six equivalents (as well as the 72 hour reaction time) was not ideal.

Previous work,<sup>64</sup> indicated that polyphosphoric acid (PPA) had been used to cause the intramolecular acetylation of pyrroles where the carbonyl functionality is an acid. The diacid [225] was refluxed with PPA but produced only baseline material. This was attributed to possible polymer formation due to the second acid group undergoing intermolecular acylation forming polymeric species or intramolecular anhydride formation. This being plausible, the mono acid [254] was investigated as a model system. Acid [254] was prepared from glycine and 2,5-dimethoxy tetrahydrofuran. This underwent efficient cyclization under conditions which failed for the diacid (Scheme 29).



Scheme 29

Having discovered that the  $\alpha$ -carboxyl function appeared to be interfering with the TMSOTf reaction it was reinvestigated on the monoester [256], the synthesis of which was attempted from the mono acid [254] and anhydrous HCl/MeOH. The expectation was to observe a small amount of the Friedel-Crafts acylation product as a by product in this reaction but in fact the acylation product [255] was the major one, in preference to [256] (Scheme 30).



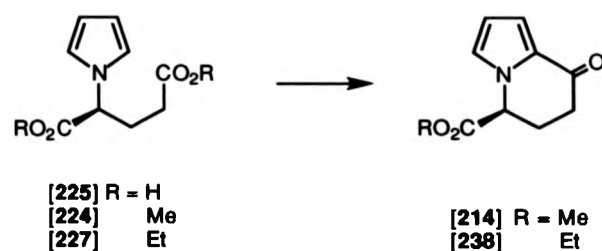
**[225]**  $\xrightarrow{\text{HCl / MeOH}}$  **[224]** (5-10%) + **[214]** (50-60%)

### Scheme 31

Finding that a simple acid could be used to cyclize this system the acidic ion-exchange resin Amberlyst 15 was studied, as the experimental conditions and work-up were expected to be very straightforward. Indeed, reflux for three days gave good yields of the desired  $\alpha$ -ketopyrrole ester.

In summary, methods have been developed for the cyclization of pyrrole diacid and diesters [225], [224] and [227] using TMSOTf, HCl/alcohol, PPA and Amberlyst 15 (Scheme 32). Table 2 compares these methods as well as that recently published by Jefford *et al*,<sup>65</sup> where cyclization is undertaken by BBr<sub>3</sub>.





Scheme 32

| Entry | Educt | Catalyst         | Product | Yield(%) |
|-------|-------|------------------|---------|----------|
| 1     | 225   | Amberlyst/MeOH   | 214     | 47       |
| 2     | 224   | HCl/MeOH         | 214     | 59       |
| 3     | 224   | TMSOTf           | 214     | 50       |
| 4     | 224   | PPA              | 214     | 24       |
| 5     | 224   | Amberlyst/MeOH   | 214     | 56       |
| 6     | 224   | BBr <sub>3</sub> | 214     | 80       |
| 7     | 227   | HCl/EtOH         | 238     | 24       |
| 8     | 227   | TMSOTf           | 238     | 13       |
| 9     | 227   | PPA              | 238     | 18       |
| 10    | 227   | Amberlyst/ EtOH  | 238     | 54       |
| 11    | 227   | BBr <sub>3</sub> | 238     | 89       |

Table 2

## 2.7 Reduction of $\alpha$ -Keto pyrroles.

### 2.7.1 Summary of new methodology

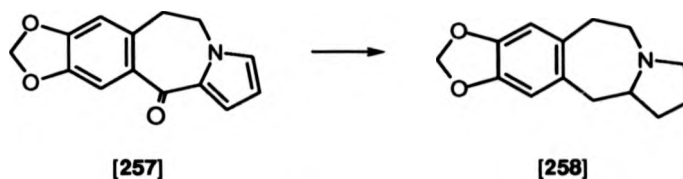
Having obtained the  $\alpha$ -ketopyrroles [214] and [238] in two steps from from L-glutamic acid [211], or its commercially available alkyl ester hydrochlorides [222] or [226], reduction of the pyrrole ring and, hopefully, concomitantly the carbonyl functionality was necessary. The use of the 5-ester functionality to direct the hydrogenation towards the opposite side of the planar ring system was anticipated. This directing effect can only be achieved using a heterogeneous system, as adsorption at a surface is needed for the hydrogen to have a large effective steric requirement. This being so, the method of choice to effect this reduction was conventional catalytic hydrogenation.

### 2.7.2 Catalytic hydrogenation.

Pyrroles are more difficult to hydrogenate than carbocyclic aromatics and the reactions are usually undertaken in acidic media. This is probably because the acid neutralizes the inhibiting effects of the amine products. Reductions may proceed stepwise through intermediate pyrrolines.<sup>94</sup>

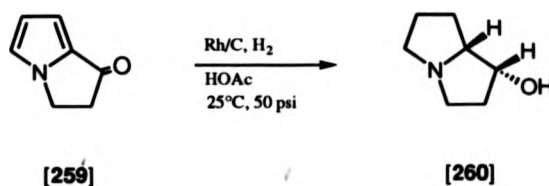
### 2.7.3 Catalysts.

A variety of catalysts have been used in the hydrogenation of pyrroles. Platinum oxide has been used often,<sup>95</sup> though for low pressure hydrogenations Rh/C or Rh/Al<sub>2</sub>O<sub>3</sub> have been found to be better, as in the reduction of [257] to [258] (Scheme 33).<sup>96</sup>



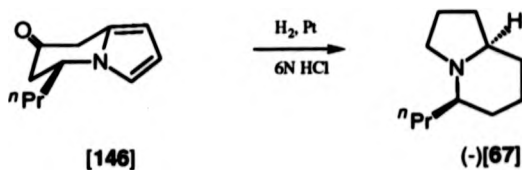
Scheme 33

However, means of selectively reducing the keto group or the pyrrole ring have proved elusive. High temperatures and pressures over nickel and ruthenium are often required for reduction and so are of limited use. Facile hydrogenation of [259] occurs over 5% Rh/C to afford [260] (Scheme 34) originally assigned as *cis*<sup>97</sup> and then later as *trans*.<sup>98</sup>



Scheme 34

Hydrogenation over platinum in 6M HCl reduces the  $\alpha$ -keto pyrrole [146] to the indolizidine (-)[67] (Scheme 35).<sup>51</sup>

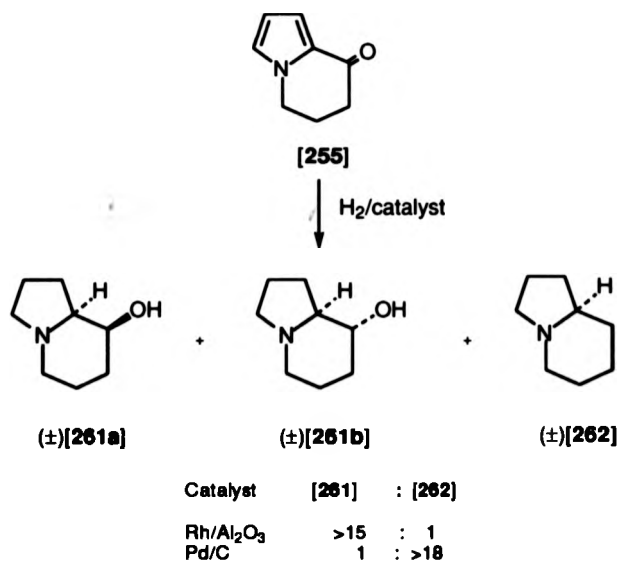


Scheme 35

Since reduction of the pyrrole system to the indolizidine and the carbonyl group to the alcohol was required, use would be made of rhodium on alumina in dry methanol in the presence of catalytic sulfuric acid.

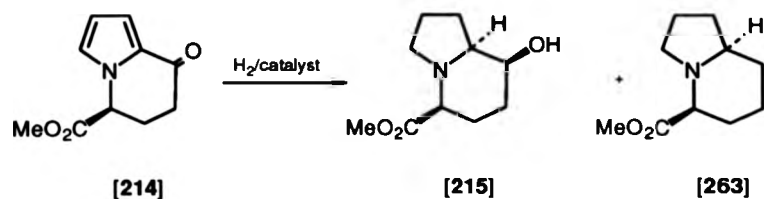
## 2.7.4 Results and discussion.

Preliminary studies were carried out on the model system [255]. Hydrogenation with rhodium on alumina gave a mixture of diastereoisomeric products ( $\pm$ )[261a] and ( $\pm$ )[261b] (which we were not able to separate completely) as well as the fully reduced compound ( $\pm$ )[262] in the ratio of >15 : 1. The catalyst was changed to Pd/C to see if one product would be favoured over the other. This also yielded a mixture of the alcohols and fully reduced products in the ratio 1 : >18 (Scheme 36).



Scheme 36

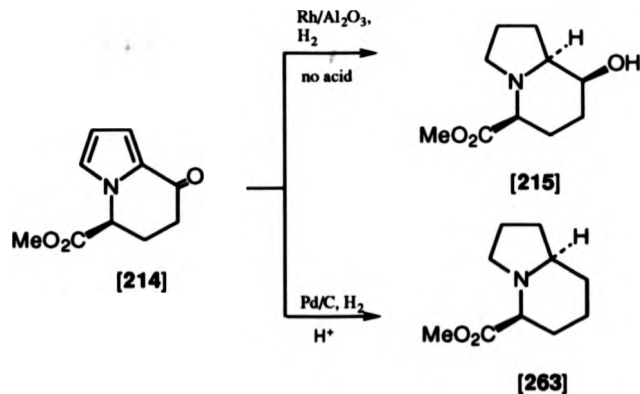
As previously discussed, the facial directing effect of the ester group in [214] was expected to introduce stereochemical control in the hydrogenation. Indeed, for both [215] and [263], only one diastereomeric product was observed, corresponding to the expected reduction from the face away from the ester group (Scheme 37).



| Catalyst      | [215] : [263] |
|---------------|---------------|
| Rh/ $Al_2O_3$ | 7 : 1         |
| Pd/C          | 1 : >10       |

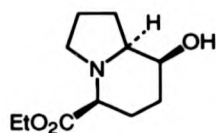
Scheme 37

Further investigations showed that using the conditions of rhodium on alumina with no acid present gave only the alcohol [215] as the sole product and palladium on carbon with acid gave only the fully reduced compound [263] (Scheme 38).

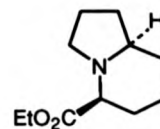


Scheme 38

By judicious choice of catalyst and presence or absence of acid, the common intermediate [214] can be used to prepare either the fully reduced indolizidine skeleton [263] or the 8(S)-alcohol [215] as the sole product.<sup>99</sup> This hydrogenation process was also extended to the ethyl esters [264] and [265].



[264]



[265]

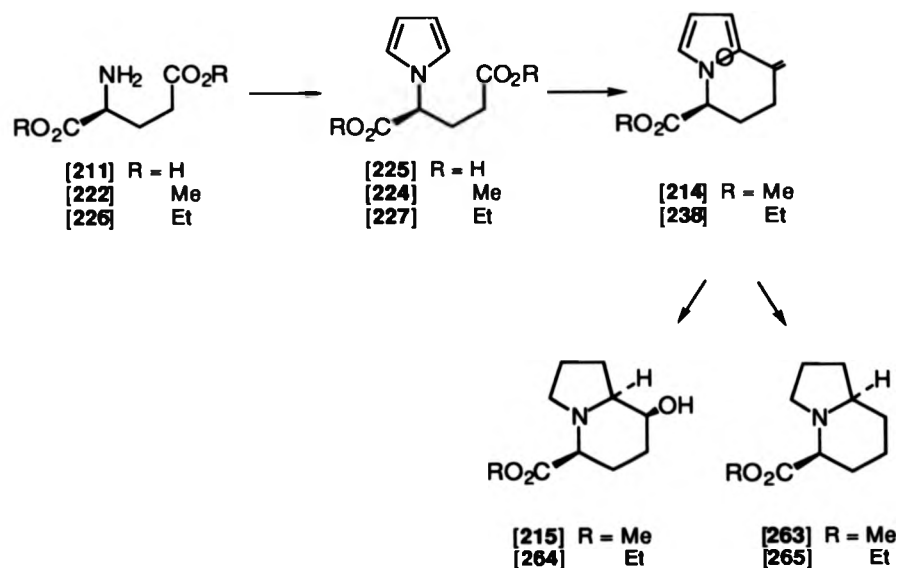
## 2.8 Hydrogenation Studies.

### 2.8.1 Introduction.

Optically pure building blocks [215], [263], [264] and [265], for the synthesis of 5-substituted and 5,8-disubstituted indolizidines were readily accessible in three steps from L-glutamic acid [211], or its commercially available hydrochlorides of the alkyl esters [222] and [226]. Palladium on carbon under acidic conditions catalyses highly diastereo- and chemo-selective hydrogenation of [214] and [238] to yield [263] and [265].

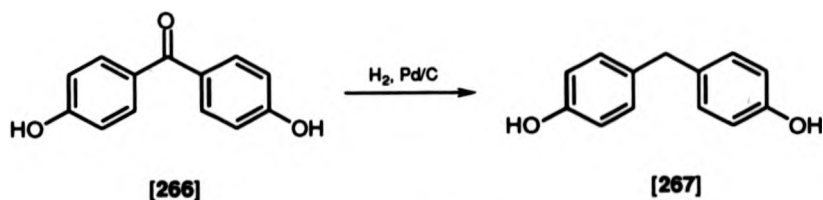
Furthermore, we have discovered that rhodium on alumina as catalyst, in the absence of acid, permits equally diastereo- and chemo-selective synthesis of the alternative hydrogenation products [215] and [264] (Scheme 39).

Although the high diastereoselectivity in the hydrogenation of compounds [214] and [238] was expected from literature precedent,<sup>51</sup> it is interesting to note the high degree of chemoselection by hydrogenation could be controlled by changing from palladium on carbon in the presence of acid to rhodium-on-alumina without acid.



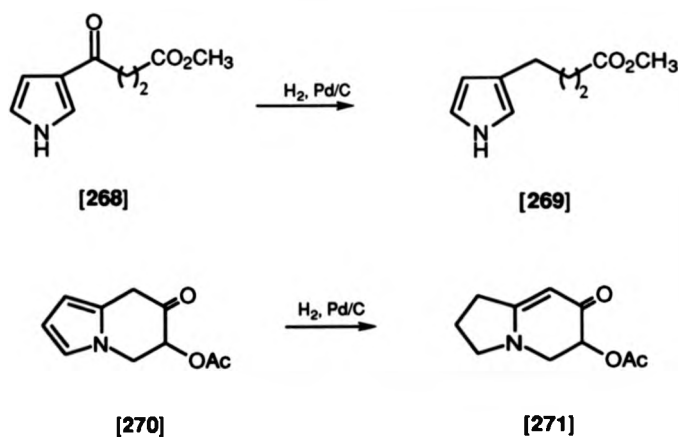
Scheme 39

Inspection of the hydrogenation products [215] and [263] indicated that they might arise from totally different reaction pathways. Palladium is known to hydrogenolyse diaryl keto groups *e.g.* *p,p'*-dihydroxybenzophenone [266] (Scheme 40).<sup>100</sup>



Scheme 40

Palladium also hydrogenolyses the keto group if it is an  $\alpha,\beta$ -unsaturated system *e.g.* [268] to [269],<sup>101</sup> whereas if the keto group is  $\beta$  to an aromatic system then the product is an  $\alpha,\beta$ -unsaturated ketone [271], prepared from the bicyclic ketone [270] (Scheme 41).<sup>102</sup>

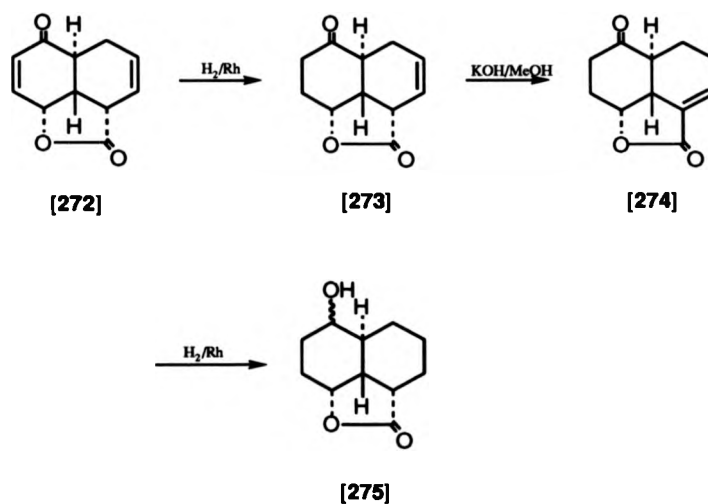


Scheme 41

Generally, palladium makes the most useful catalyst for hydrogenolysis of aromatic ketones, as it combines a good activity for hydrogenation and hydrogenolysis of the carbonyl with low activity for hydrogenation of the aromatic nucleus.<sup>100</sup>

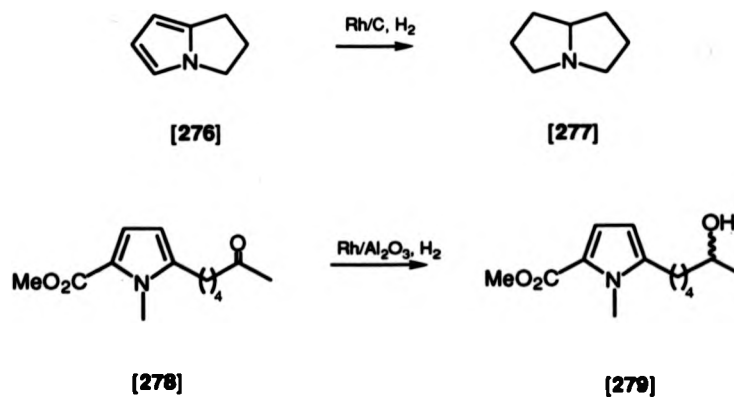
Roy and Wheeler<sup>103</sup> showed that in the  $\alpha,\beta$ -unsaturated lactone [272], hydrogenation with rhodium as the catalyst yielded the dihydro derivative [273], showing preferential hydrogenation of the double bond with respect to the keto-functionality. Treatment with methanolic KOH effected isomerization of the double bond to give the  $\alpha, \beta$  unsaturated lactone [274] and subsequent hydrogenation over rhodium yielded the hydroxy lactone [275], (Scheme 42).

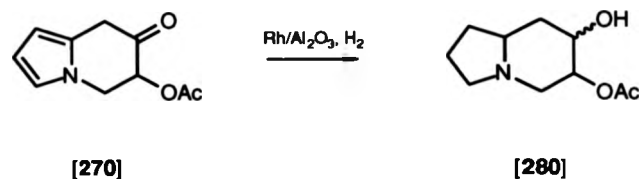




Scheme 42

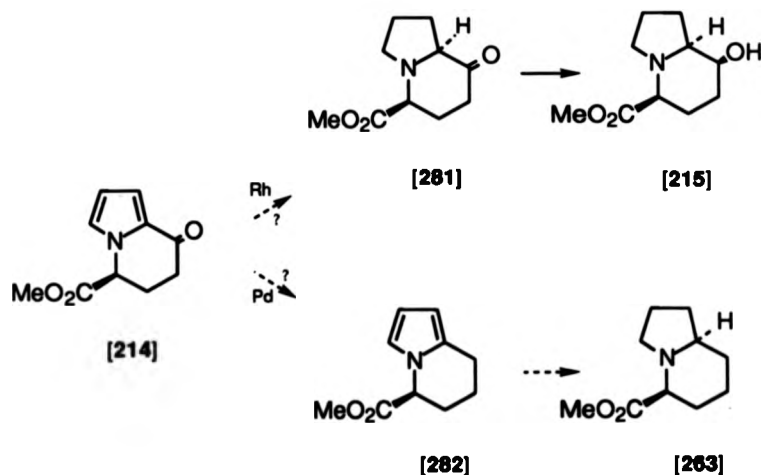
In the field of pyrrole chemistry both rhodium on carbon and rhodium on alumina have been used; rhodium on carbon to catalyze the reduction of the bicyclic pyrrole [276] to pyrrolizidine [277]<sup>104</sup> and rhodium on alumina to catalyze the reduction of the ketone [278] to the alcohol [279].<sup>105</sup> Jefford<sup>102</sup> used rhodium catalysed hydrogenation to affect the full reduction of the 5,6-bicyclic ketone [270] to the substituted indolizidine [280] (Scheme 43).





Scheme 43

A possible explanation is that during hydrogenation of the  $\alpha$ -keto pyrrole [214] with palladium, the ketone, while in conjugation with the pyrrole, was reduced to the methylene compound [282] with subsequent hydrogenation of the pyrrole yielding the indolizidine [263]. With rhodium, reduction of the pyrrole might occur first, the non-conjugated ketone [281] would then be unlikely to be hydrogenated to the indolizidine [263] but would instead proceed to the corresponding alcohol [215] (Scheme 44). To test this hypothesis the proposed intermediates were prepared ([281] and [282]) and submitted to hydrogenation conditions.

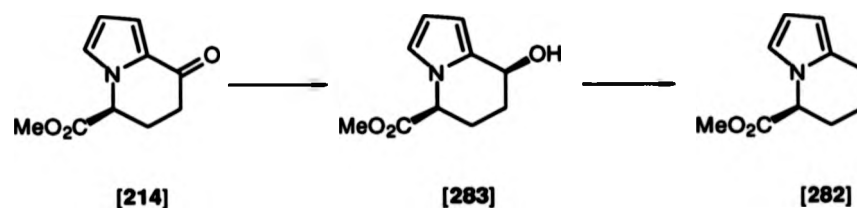


Scheme 44

## 2.8.2 Results and discussion.

Swern oxidation<sup>106</sup> of alcohol [215] proceeded smoothly to yield the ketone [281]. This was ascertained both by  $^1\text{H}$  and  $^{13}\text{C}$  NMR. Both the alcohol resonance and the C(8)  $\text{H}$  resonance at  $\delta = 3.78$  ppm in the  $^1\text{H}$  NMR were absent in relation to the starting material. In the  $^{13}\text{C}$  NMR the appearance of a second resonance in the carbonyl region, along with that of the methyl ester, confirmed that oxidation had taken place. Due to the unstable nature of the product, no further analytical evidence was gathered and the crude ketone was used in the hydrogenation reactions with no further purification.

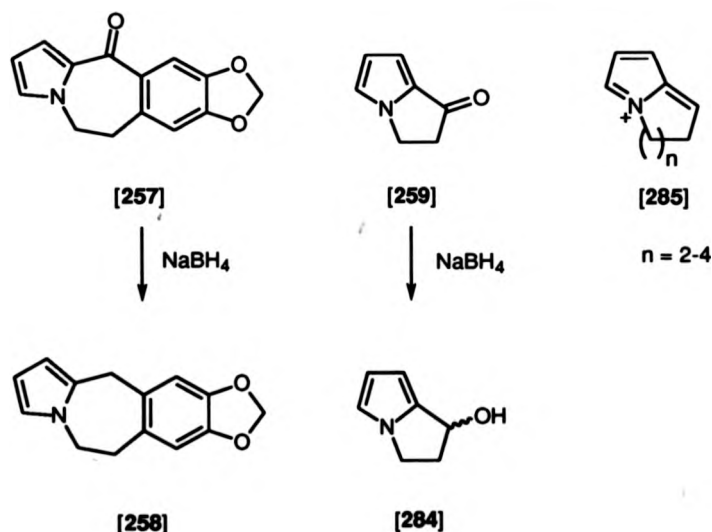
As alcohol [283] was also an attractive substrate for the studies, it was prepared from the ketone [214]. However, borane/dimethylsulfide reduction<sup>107</sup> of the ketone [214] was found to proceed directly to the methylene analogue [282] (Scheme 45), as indicated by the absence of a hydroxyl stretching frequency in the IR. This did, however, contain the expected peaks arising from the pyrrole system. There was no evidence of the proposed alcohol in the  $^1\text{H}$  NMR and integration in the region for the methylene envelope assigned six protons for this region, for the C(6-8) $\text{H}$ s. The  $^{13}\text{C}$  NMR showed the expected pattern for the 1,2-disubstituted pyrrole, the remaining signals were consistent with the reduced pyrrole [282].



Scheme 45

Decomposition meant that CHN measurements were inaccurate and so are not included in the experimental.

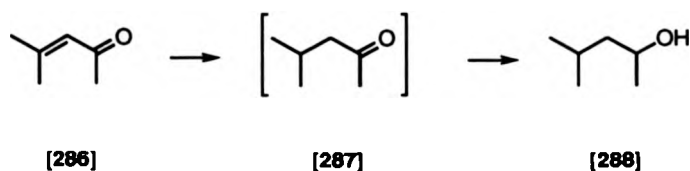
Ketone to methylene reductions have also been observed for sodium borohydride reduction of a seven-membered analogue [257],<sup>96</sup> but reduction of the five-membered analogue [259] did stop at the alcohol stage.<sup>97</sup> These results can be rationalised if it is assumed that the second step of the reduction (CHOH to CH<sub>2</sub>) proceeds *via* intermediate iminium ions [285].<sup>108</sup> In the case of the five-membered analogue [259], strain would prohibit formation of the iminium ion (Scheme 46).



Scheme 46

The proposed intermediate [281] was not affected by the usual hydrogenation conditions with either catalyst. The other proposed intermediate [282] was partially reduced to the indolizidine [263] with both rhodium-on-alumina (no acid) and palladium-on-carbon (with H<sub>2</sub>SO<sub>4</sub>) but much more slowly than with the keto pyrrole [214] as substrate. Therefore neither of the proposed intermediates ([281] and [282]) are likely intermediates

in the hydrogenation of the keto pyrrole [214] with either catalyst system. This conclusion differs from that of Breitner *et al.*<sup>109</sup> They carried out studies to compare Pt, Pd, Rh and Ru as hydrogenation catalysts. They found that in the reduction of mesityl oxide [286] to the saturated alcohol [288], reduction proceeded solely through the saturated ketone [287] (Scheme 47). The availability of the pyrrole lone pair to the conjugated system is clearly of significance.

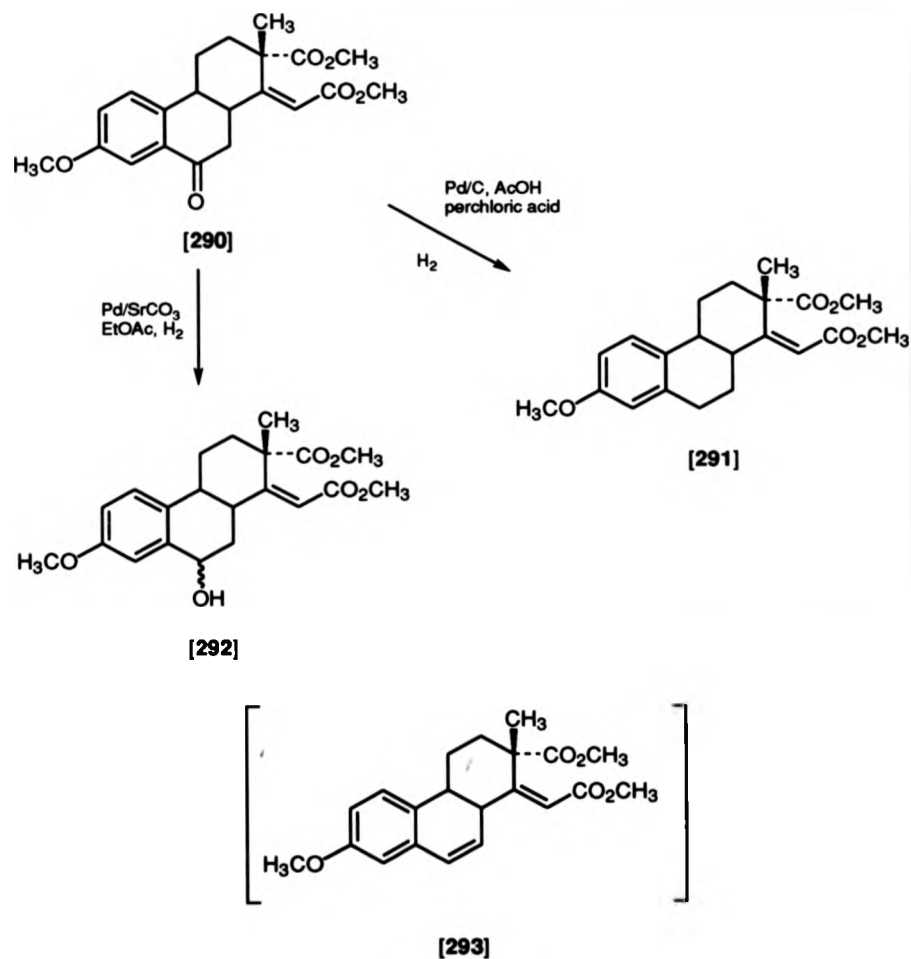


Scheme 47

The discovery that the pyrrole [282] was reduced to the indolizidine [263] much more slowly than the keto pyrrole [214], along with the discovery that an iminium ion is a probable intermediate in the facile reduction of the ketone [214] to the alcohol [215], led to proposition of the mechanism shown in Scheme 48.

With both catalysts, the first step is reduction of the ketone [214] to yield the alcohol [283]. This can undergo either reduction of the pyrrole to produce the hydroxy indolizidine [215] or elimination to produce the iminium ion [289], the latter subsequently being further reduced to produce the reduced indolizidine [263]. From the observed data it can be concluded that  $k_1$  is greater with rhodium on alumina than with palladium on carbon. The rate of elimination  $k_2$  will be accelerated in the presence of acid. This hypothesis is consistent with the observations that mixtures of hydroxy indolizidine [215] and indolizidine [263] result both (a) when rhodium on alumina is used with





Scheme 49

The proposed intermediate [293] has a conjugated double bond and a non-conjugated double bond. The conjugated bond, being more susceptible to hydrogenation, would be reduced *in situ* yielding the hydrogenolysis product [291].

## 2.9 Structural elucidation of Indolizidines.

### 2.9.1 Infra-red spectroscopy.

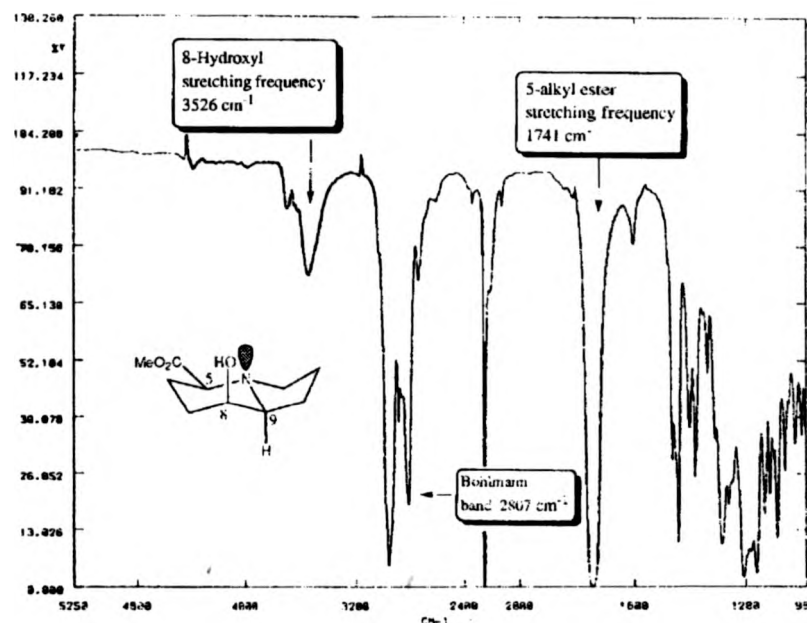
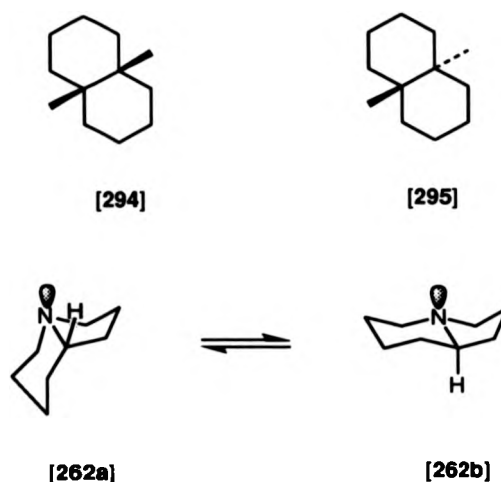


Figure 6.  $^1\text{H}$  NMR spectrum of Indolizidine [215].

Infra-red (IR) spectroscopy of the indolizidine [215], (Figure 6) showed an absorption at  $3526\text{ cm}^{-1}$  that was attributed to a hydroxyl group. Another at  $1741\text{ cm}^{-1}$  was assigned as the C=O stretch of the ester. It was therefore deduced that both the functionalities we required were in the molecule though their position had yet to be ascertained. IR also gives information on the fusion of the piperidine and the pyrrolidine rings. Although *cis*- and *trans*- fused hydrindanes ([294], [295]) (bicyclo[4.4.0]decane), the carbocyclic analogues of the indolizidines, are well known,<sup>112</sup> the *cis*- and *trans*- isomers of indolizidine [262] cannot be isolated, as rapid inversion of configuration at nitrogen interconverts them, producing an equilibrium mixture (Scheme 50).



A *trans*-linkage of the rings gives rise to Bohlmann bands<sup>113</sup> in the I.R. spectrum.



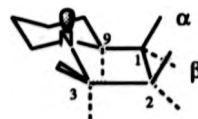
Scheme 50

The Bohlmann bands occur at *ca* 2800  $\text{cm}^{-1}$  and are brought about when two or more  $\alpha$ -hydrogens are *trans*-diaxial to the lone pair on nitrogen. The spectra obtained show an absorption at 2807  $\text{cm}^{-1}$ , thus indicating that the *trans*- [262b] is present, although this does not exclude the presence of the *cis* conformer [262a].

The fact that indolizidines are significantly more stable with a *trans*-ring fusion has been shown by modeling and by spectroscopic determination of  $-\Delta G^\circ$ , where Aaron *et al*<sup>114</sup> found that in 8-hydroxyindolizidine, the total *cis*-fused population was only 4%. The free energy difference between the *cis*- and *trans*-fused conformers was -2.4  $\text{kcal mol}^{-1}$  which indicates a strong preference for the *trans* conformation.

### 2.9.2 NMR spectroscopy.

Basing their investigation on the IR data for the parent *trans*-fused indolizidine, Ringdahl *et al* <sup>115</sup> went on to prove the *trans* ring fusion from the 360 MHz <sup>1</sup>H NMR data. In comparing indolizidine with indolizidine hydrobromide (Figure 7), they used coupling constants from the spin-decoupled and expanded spectra to put forward the structure of a *trans*-fused ring system with a chair shaped piperidine and an envelope conformation for the pyrrolidine ring, the nitrogen being displaced out of the plane formed by C(1), C(2), C(3) and C(9). This is in agreement with the X-ray crystallographic data of some indolizidine alkaloids investigated by Daly *et al* <sup>26</sup> with the carbon atoms 1,2,3 and 9 essentially coplanar (within  $\pm 0.17$  Å) and the nitrogen atom puckered out of the ring [262]. Normally, the terms 'axial' and 'equatorial' are used when discussing substituents in six membered rings indolizidine [262] has been drawn in such a way as to illustrate that the C(3) substituents can be approximated to axial and equatorial though for those on C(1) and C(2) the labels  $\alpha$  and  $\beta$  are more appropriate ( $\alpha$  being on the side of the nitrogen lone pair,  $\beta$  on the opposite) as they bisect the plane.<sup>116</sup> The terms pseudo-equatorial and pseudo-axial are also commonly used.



[262]

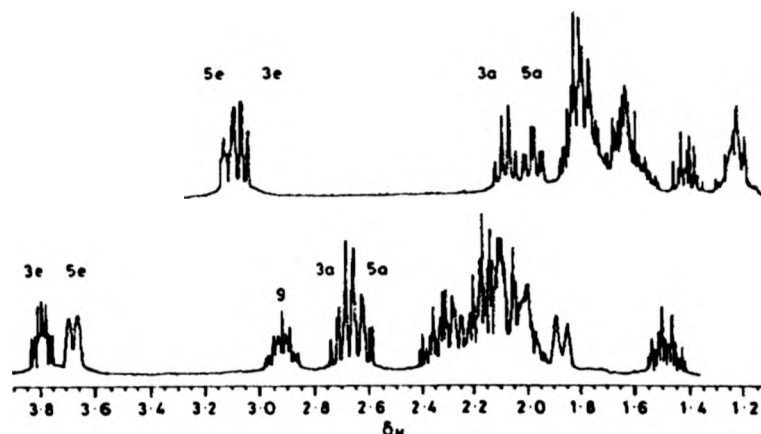


Figure 7.  $^1\text{H}$  NMR spectrum on indolizidine (upper) and indolizidine hydrobromide (lower) in  $\text{CDCl}_3$ <sup>115</sup>

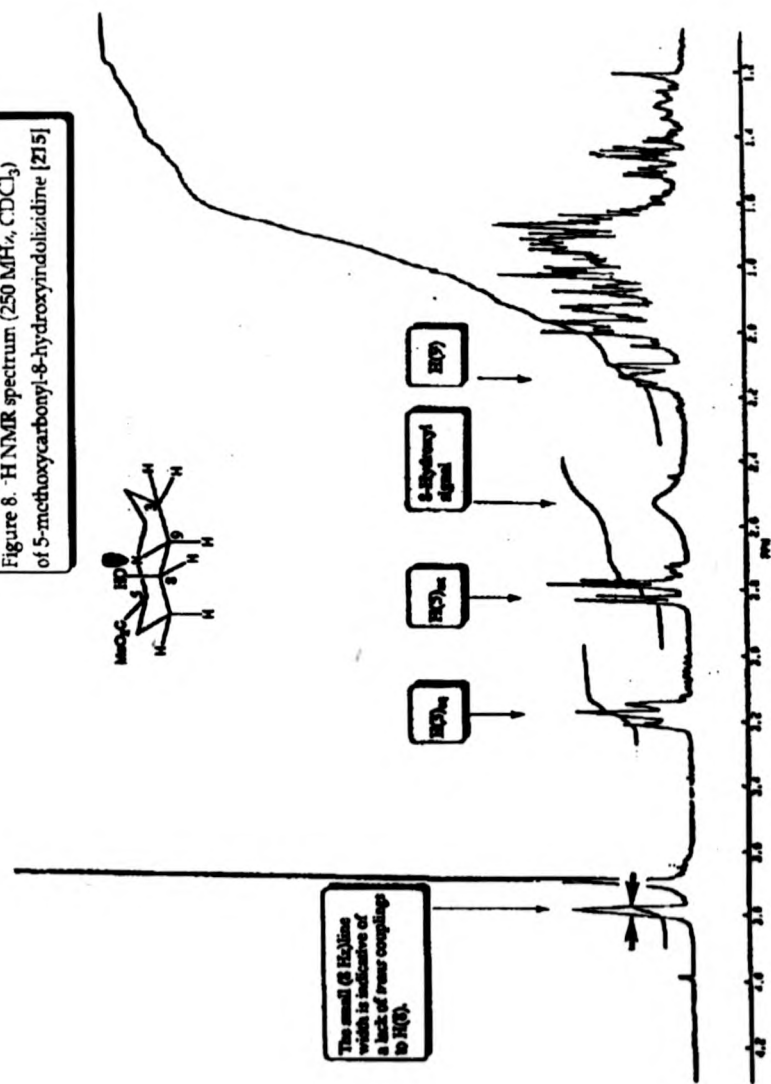
In our  $^1\text{H}$  spectra of 5-methoxycarbonyl-8-hydroxyindolizidine [215] (Figure 8) the peak at  $\delta$  3.8 ppm integrates to one proton and has been assigned (using  $^1\text{H}$ ,  $^{13}\text{C}$ , C-H correlation, COSY and NOE) as C(8)H. It is an unresolved multiplet which appears as a broad singlet with a line width of 8 Hz at half-height. Its shift upfield with respect to the C(8)H resonance in the methylene envelope of the parent indolizidine is due to the hydroxyl group also on C(8).  $^3\text{J}$  couplings H-H<sub>trans</sub> are of the order of 12-15 Hz, H-H<sub>cis</sub> are of the order of 6-8 Hz. The proton on C(8) must therefore be equatorial to give all *cis* couplings as there are no couplings greater than 8 Hz in this multiplet. If this proton was axial then the  $^3\text{J}$  C(9)H-C(8)H and C(8)-C(7)H<sub>ax</sub> couplings would make the line width broader than the observed 8 Hz. As C(8)H is equatorial then C(8)OH must be axial giving the C(8) position the (*S*)-configuration

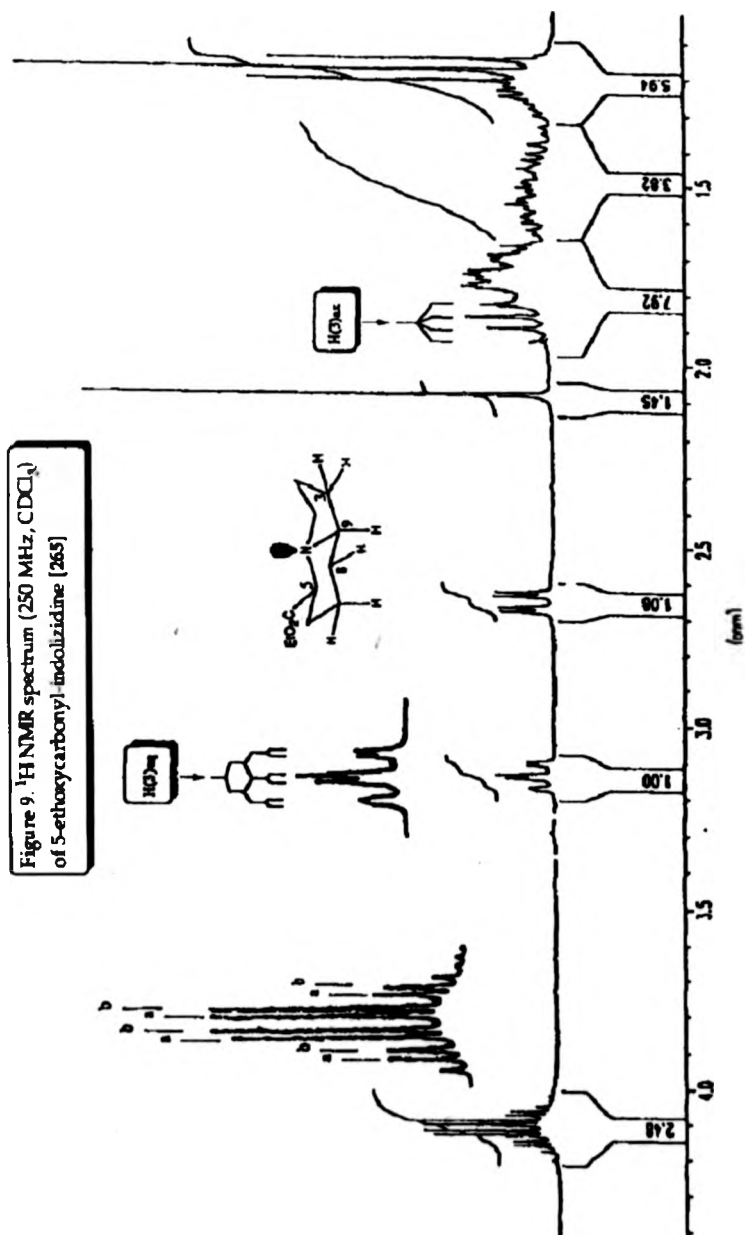
The singlet at  $\delta$  3.68 ppm integrates to three protons and was assigned to the methyl ester protons. The distorted triplet at  $\delta$  3.18 ppm is assigned as H(3)<sub>eq</sub> in accordance with the parent indolizidine.<sup>115</sup> The absence of the

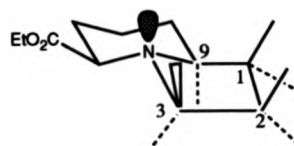
H(5)<sub>eq</sub> resonance indicated that the 5-ester moiety was equatorial, not axial, the doublet of doublets at  $\delta$  2.8 ppm being assigned as H(5)<sub>ax</sub>. This was deduced in comparison with 5-cyanoindolizidine where the nitrile group (which has a similar effect on chemical shift to an ester group) was in an axial position and the H(5)<sub>eq</sub> had a shift of  $\delta$  4.66 ppm. A similar shift would be expected if H(5) were in fact equatorial in our case. The significantly upfield resonance at  $\delta$  2.8 ppm was assigned as H(5)<sub>ax</sub>. nOe measurements showed an nOe of 3.5% for the H(5)<sub>ax</sub>-H(9) interaction and 2.5% for the H(8)<sub>eq</sub>-H(9) interaction. These observations therefore confirmed the 5(R), 8(S) assignments. The other resonances of interest in (Figure 8) are the hydroxyl resonance at  $\delta$  2.52 ppm and the H(9) resonance brought out of the methylene envelope to appear at  $\delta$  2.21 ppm.

This information gives little or no indication of the conformations of the molecule. For this the spectrum of 5-ethoxycarbonylindolizidine [265] (Figure 9) was utilized. The equatorial proton H(3)<sub>eq</sub> appears as a doublet of triplets. The positions of the carbon atoms C(1) and C(3) are fixed if it is assumed that the piperidine ring is in a chair conformation.<sup>26</sup> The pattern observed for H(3)<sub>eq</sub> suggests that the methylene hydrogens on C(2) and C(3) are nearly eclipsed and that C(2), C(3) and C(9) are virtually co-planar. The multiplicity can be explained as arising from the geminal coupling and coupling to one of the vicinyl methylene protons of a similar magnitude (*ca* 8 Hz), as well as a smaller coupling (2.6 Hz) to the other methylene proton.

Figure 8.  $^1\text{H}$  NMR spectrum (250  $\text{MHz}$ ,  $\text{CDCl}_3$ ) of 5-methoxycarbonyl-8-hydroxyindolizidine [215]







[265]

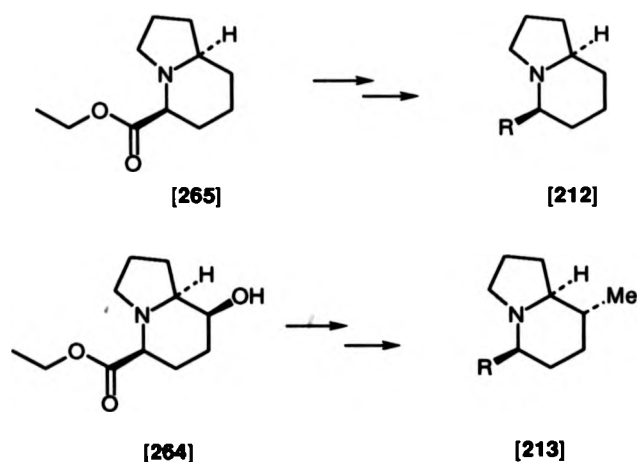
The axial proton  $H(3)_{ax}$ , appears as a quartet indicating that the geminal and two vicinal coupling constants are of a similar magnitude. This can only arise if C(1), C(2), C(3) and C(9) are nearly co-planar. This gives dihedral angles between  $H(3)_{ax}$  and the C(2) methylene protons of *ca* 30 and 150°. This is in good agreement with the assignments in indolizidine<sup>115</sup> and X-ray data on Daly's indolizidine alkaloids.<sup>26</sup>

**Substitution at the 5- and 8-positions.  
Chapter: 3**



### 3.1 Introduction.

Having obtained the enantiomerically pure (5*S*,9*R*)-5-ethoxycarbonyl-indolizidine [265] and (5*S*,8*S*,9*R*)-5-ethoxycarbonyl-8-hydroxyindolizidine [264] the aim was to convert these substituted indolizidines to the required, natural, 5-alkyl indolizidines [212] and 5-alkyl-8-methyl indolizidines [213] as well as some simple analogues (Scheme 51).



Scheme 51

As stated in Ing's 5-chain rule<sup>46</sup>, for muscarinic activity there should be five chain atoms linking a tertiary nitrogen and the chain terminus. This criteria is fulfilled with the 8-methyl group on the indolizidine ring system. It was necessary to convert the 8*S* hydroxyl group to the required 8*R* methyl group. The obvious solution was to activate the alcohol with a moiety that would provide a good leaving group when an  $S_N2$  attack of  $Me^-$  was undertaken. This would invert the stereochemistry at C(8) yielding the desired 8*R* methyl group. This methodology could also be applied to chain extension at the 5-position. A reduction of the 5-ester to the alcohol was envisaged, then activation of the alcohol as the tosylate followed by nucleophilic displacement with  $R^-$ .

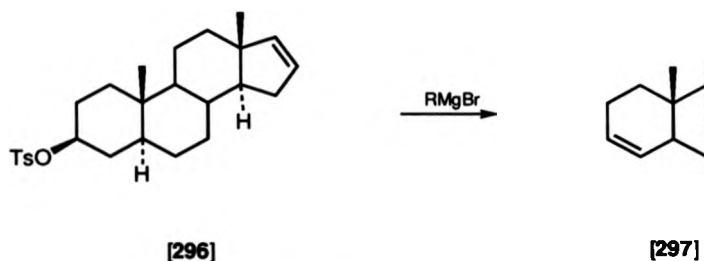
## 3.2. Manipulation of the 8-substituent.

### 3.2.1 Activation of the alcohol.

Nucleophilic substitution of hydroxyl groups requires prior activation of the C-O bond. Sulfonation is the most common approach used to achieve this. Alkane and arenesulfonic esters are important reagents in modern synthetic chemistry and are prepared from the corresponding sulfonyl chlorides and an alcohol.<sup>117</sup> In general mesylates (methanesulfonic esters) are more reactive than tosylates<sup>118</sup> (*p*-toluenesulfonic esters). The more recently developed triflates (trifluoro analogues) are even more reactive.<sup>119</sup> The order of stability is Ts>Ms>Tf.

### 3.2.2 Grignard Reagents.

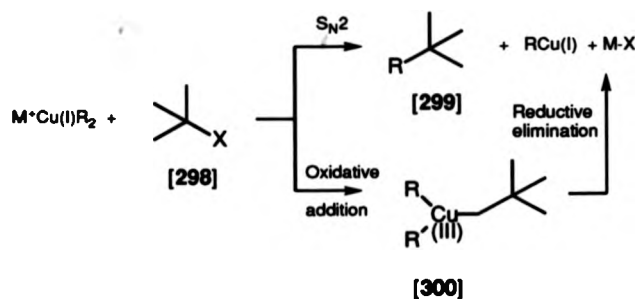
Grignard reagents have the advantage over organocuprates in that they are simpler to prepare. However, the scope of Grignard mediated reactions is much narrower than those involving the corresponding organocuprates. Grignard reagents couple only with activated halides (allyl and benzylic) and tosylates, with the aryl Grignard reagents giving superior yields compared to the alkyl. Grignards are tolerated by a number of functional groups, *e.g.* ethers, but will react with carbonyl and carboxyl groups to produce alcohols and ketones, respectively. As the compounds [264] and [265] contain the 5-ester functionality Grignard reagents would not seem to be ideal. Another consideration of Grignards is that where a tosyl group is present, often the elimination product rather than the desired substitution product results, for example [297] is produced from Grignard attack on [296] (Scheme 52).<sup>120</sup>



Scheme 52

### 3.2.3 Organocuprates.

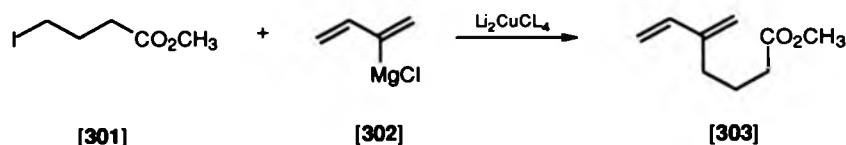
Organocuprates are among the most versatile reagents in organic synthesis.<sup>121,122</sup> Indeed, copper is probably the most widely used of the transition metals in the field of organometallic and organic synthesis for the construction of C-C bonds.



Scheme 53

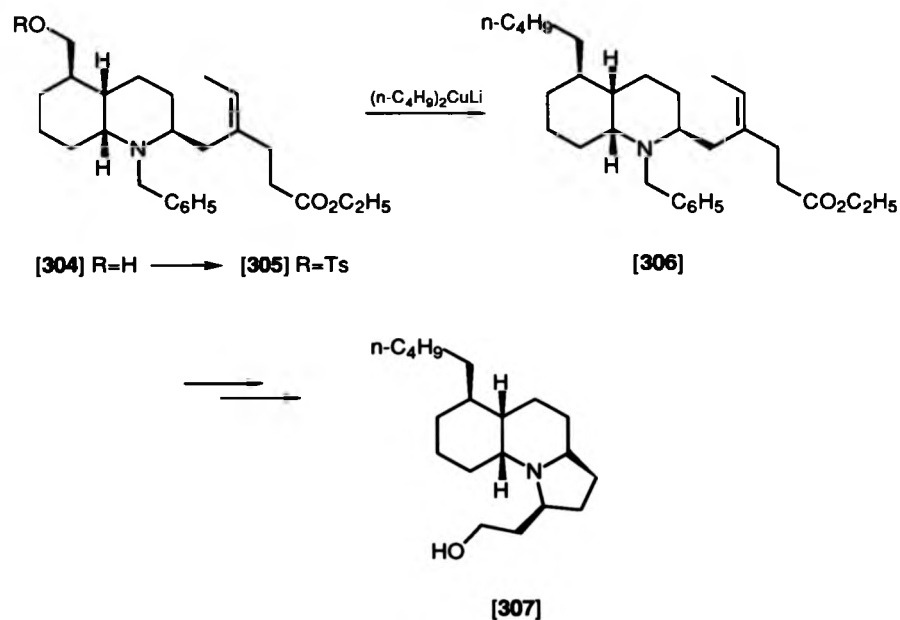
The proposed mechanism for substitutions of halides and sulfonates is usually the direct displacement by R of  $R_2CuLi$ . This fulfills the criteria of an  $S_N2$  type substitution (though an oxidative addition/reductive elimination mechanism is sometimes involved the reaction is still, overall, a substitution reaction) (Scheme 53).<sup>123</sup>

Tosylates<sup>124</sup> and epoxides<sup>123</sup> both react with organocuprates with inversion. More recent work has shown that not all halide displacements proceed with inversion of configuration at the carbon center<sup>125,126</sup> This being the case, it would seem that tosylate is more reliable than halide as the species for our desired inversion at C(8) *via* the S<sub>N</sub>2 substitution. Another advantage of the organocuprates is the relative inertness to other functionalities, especially towards esters (Scheme 54).<sup>127</sup> This is the case using either the catalytic Li<sub>2</sub>CuCl<sub>4</sub> species or the more widely used 'R<sub>2</sub>CuLi' complex in stoichiometric amounts.



Scheme 54

Several syntheses have been undertaken in the alkaloid area with the strategy detailed above, *viz.* activation of the alcohol as a tosylate and displacement with a lithiocuprate, for example in the synthesis of perhydrogephyrotoxin [307] (Scheme 55).<sup>128</sup>



Scheme 55

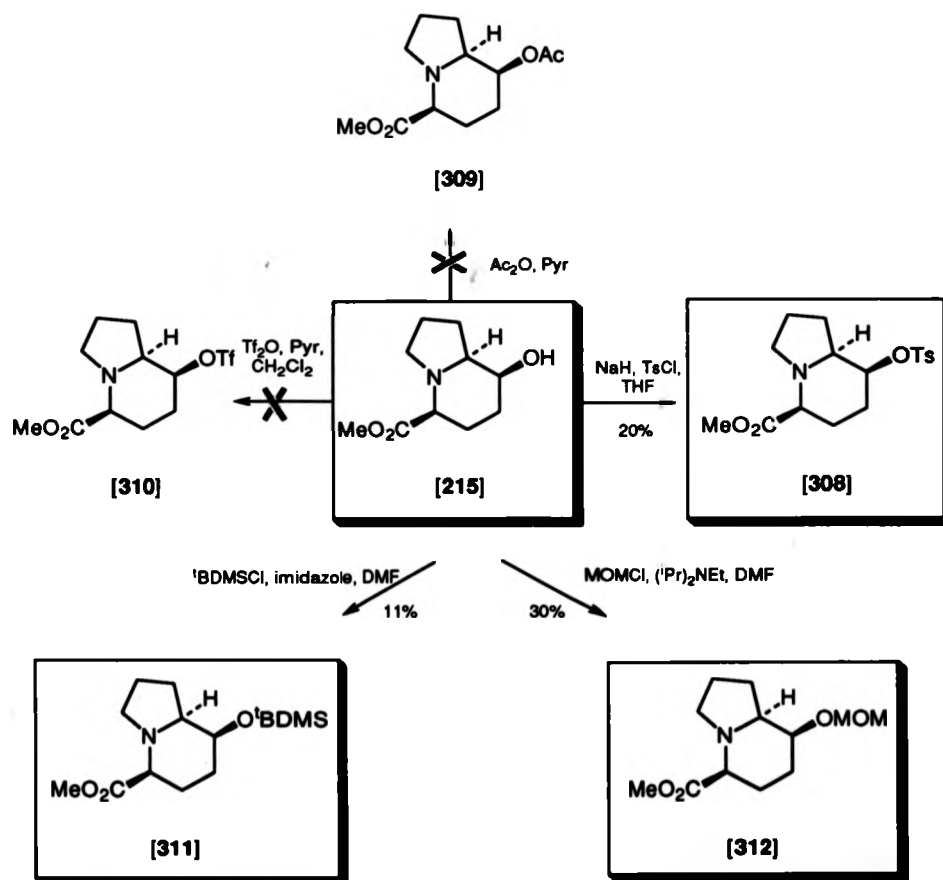
### 3.2.4 Results and Discussion. Activation of the 8-hydroxyl group.

Tosylation was first attempted under standard conditions, *viz.* tosyl chloride in dry pyridine.<sup>129</sup> Surprisingly this only gave a yield of *ca* 20% of the tosylate [308] and recovery of starting material. Other conditions were tried, such as tosyl chloride in triethylamine,<sup>130</sup> and with catalytic amounts of DMAP (4-dimethylamino pyridine).<sup>131</sup> All of these conditions gave very low yields, so other activating groups were considered. Acetylation and triflation both failed giving no product or starting material, but base-line material on TLC (Scheme 56).

To establish whether the failure of these reactions was due to low reactivity or steric hindrance of the alcohol, an attempt was made to alkylate and

silylate with MOMCl and <sup>t</sup>BDMSCl respectively. These reactions were selected as the products are protected alcohols which might be useful in further studies. Reaction of alcohol [215] with MOMCl<sup>132</sup> yielded the MOM ether [312] in 25% yield (Scheme 56).

Molecular modeling showed that there should be no steric barrier to the protection of the 8-hydroxyl group. Indeed alcohol [215] did react with <sup>t</sup>BDMSCl to yield the protected alcohol [311], although the yield was again low (11%) (Scheme 56). Trimethylsilyl protection is discussed in section 3.2.5.



Scheme 56

The low yields in the reactions of alcohol [215] with the various electrophiles was surprising, as Overman *et al*<sup>128</sup>, in the synthesis of perhydrogephyrotoxin [307] and Holmes *et al*<sup>48</sup> in the synthesis of (-)-209B [76] found the mesylate to pose no problems. It is possible that the problems lay in the position of the alcohol group  $\beta$  to the tertiary nitrogen.

Another factor may be that the combined equatorial preferences of the ester substituent and the five-membered ring would force the tosyl group to be axial in a chair conformation (Figure 10), this being unfavourable. The problematic tosylation reaction will be further discussed in section 3.4.

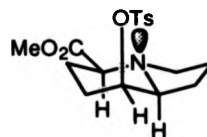


Figure 10. Tosyl activated indolizidine [308].

### 3.2.5 Results and Discussion. <sup>t</sup>BDMSCl protection of the 8-hydroxyl group.

As well as the reaction of [215] with <sup>t</sup>BDMSCl (with imidazole in DMF)<sup>129</sup> to give the <sup>t</sup>BDMS protected alcohol [311], protection of the 8-alcohol as the trimethylsilyl derivative, using TMSCl and sodium hydride in THF as the solvent was attempted.<sup>130</sup> This produced a white solid [313]. No hydroxyl peak was seen in the IR but the <sup>1</sup>H NMR did not correspond to what was expected. In particular, no TMS singlet was observed.

### 3.2.6 Characterisation of the unknown compound [313].

The IR spectrum (Figure 11) showed that there was no hydroxyl group and that the carbonyl absorption had shifted from  $1741\text{cm}^{-1}$  in the starting material to  $1751\text{cm}^{-1}$  in the product. This is not a large difference but this, in combination with strong absorptions at  $1270$  and  $1260\text{cm}^{-1}$ , indicated either an ester or a  $\delta$ -lactone.

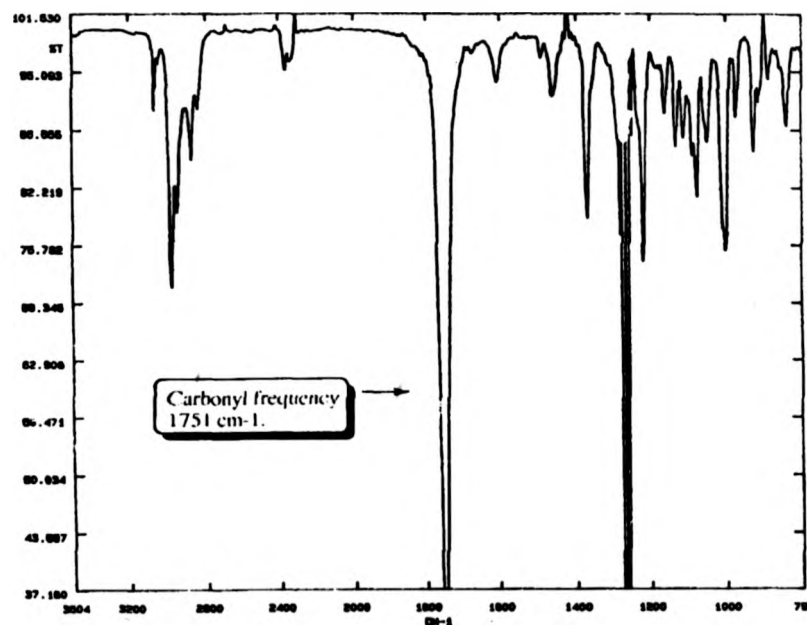
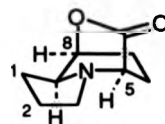


Figure 11. IR spectrum of unknown compound [313].

Integration of the  $^1\text{H}$  NMR spectrum (Figure 12) and the number of resonances in the  $^{13}\text{C}$  spectrum gave a ratio of C:H of 9:13. This, coupled with the idea that lactonisation might have occurred led us to postulate the structure [313].





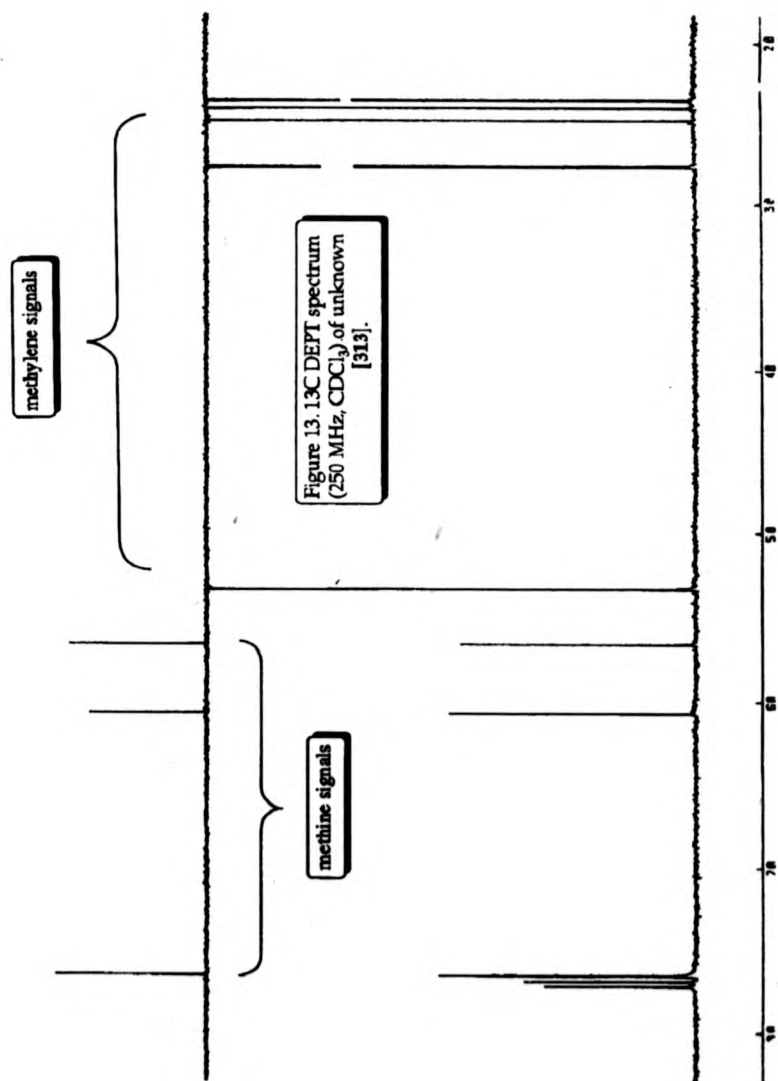
[313]

The  $^{13}\text{C}$  DEPT spectrum (Figure 13) showed that there were five methylene and three methine carbons. From the  $^1\text{H}$ - $^{13}\text{C}$  correlation spectrum it was simple to assign the  $^1\text{H}$  resonances at  $\delta$  3.27, 3.34 and 4.75 ppm as methine signals due to H(9), H(5) and H(8), respectively. As described in section 2.9.2 it is possible to distinguish equatorial and axial protons by the lack of significant coupling in the former. Thus, H(5) and H(8), which appear as broad singlets, must be equatorial whereas H(9), which is a partly resolved multiplet, is axial. This is important in confirming the tricyclic structure [313] which forces the piperidine ring into a boat conformation.

The resonance at  $\delta$  3.27 ppm was assigned as  $\text{C}(3)\text{H}_{\text{ax}}$  and appeared as an apparent triplet. As in  $\text{C}(3)\text{H}_{\text{eq}}$  of 5-ethoxycarbonyl indolizidine [265] the argument follows that the geminal and one of the vicinal couplings are of a similar magnitude, though in this case the second, smaller vicinal coupling is unresolved. The complex multiplet at  $\delta$  2.53 ppm is assigned as  $\text{C}(3)\text{H}_{\text{eq}}$  and indicates, from its multiplicity, in conjunction with the resonance multiplicity for  $\text{C}(3)\text{H}_{\text{ax}}$ , that the puckered envelope configuration of the pyrrolidine ring with the nitrogen out of the plane is no longer present. The rest of the methylene protons are contained in the multiplet  $\delta$  2.1-1.6 ppm.

The high resolution mass spectrum and  $\text{C,H,N}$  analysis were also consistent with the proposed product [313].





### 3.3 Manipulation of the 5-substituent.

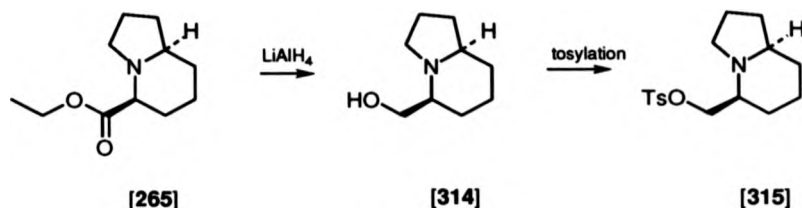
#### 3.3.1 Summary of Methodology: Homologation through activation/substitution.

There is literature precedent for the activation of hydroxyl groups with tosyl chloride and subsequent substitution using lithiocuprates. This methodology has been used in structurally related compounds including indolizidines.<sup>48</sup> It was envisaged that application of this methodology would lead to the 5,8-disubstituted indolizidines.

Simple analogues of the natural products were also planned by varying the nature of the 5-ester group, as discussed in Section 3.3.4.

#### 3.3.2 Results and Discussion. Side-chain hydroxyl group activation.

Ester [265] was first reduced to the alcohol [314] using  $\text{LiAlH}_4$  under standard conditions (anhydrous THF under an inert atmosphere)(Scheme 57).<sup>131</sup> The alcohol [314] was isolated in 70% yield and required no further purification. Tosylation of the alcohol [314] was more problematic. No tosylate [315] was seen under standard conditions. Even with more forcing conditions using  $n\text{BuLi}$  as base, the tosylate [315] was only obtained in 26% yield. In view of the difficulty of obtaining the tosylate [315], bromination was instead attempted. However, reaction with  $\text{CBr}_4$  and  $\text{PPh}_3$ <sup>132</sup> led to a complex mixture of products and this route was therefore abandoned.



Scheme 57

As elimination products appeared to have resulted from the attempted synthesis of the bromide, activating groups which were more reactive than tosyl *e.g.* mesyl, triflate and nonanflate, were not investigated as it was thought that these would also readily eliminate. The low yields found in these reactions may be, as with the 8-hydroxyl group, due to the  $\beta$ -position of the alcohol with respect to the tertiary amine, (see section 3.4).

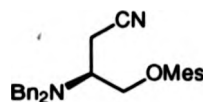
### 3.3.3 Results and Discussion. C-5 chain extension.

Reaction of the tosylate [315] with lithiocuprates or Grignard reagents failed to produce the desired products. Instead work-up (saturated aqueous ammonium chloride and extraction with ether) yielded only starting material in all cases. The reaction was performed with both  $\text{Me}_2\text{LiCu}$  and  $n\text{Bu}_2\text{LiCu}$  by addition of the lithiocuprate to a solution of the tosylate. The reverse addition was also investigated with the same result. Likewise, addition of a Grignard reagent to a solution of the tosylate resulted in only starting material being isolated.

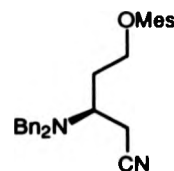
During the reaction, there was indication that the lithiocuprate had formed due to the colour changes observed. A dark yellow precipitate was formed on addition of one equivalent of the alkyllithium to the copper (I) iodide and a deep green/black solution was formed upon the addition of the second

equivalent. The Grignard reagent was taken from a fresh bottle ordered from the Aldrich Chemical company which contained no deposit of magnesium salts. Four equivalents of both the lithiocuprate and Grignard reagent were used at a temperature of  $-20^{\circ}\text{C}$  with reaction times in excess of 18 hours but none of the desired product was obtained. There are other examples of  $\beta$ -amino groups retarding coupling reactions in this way.<sup>133</sup>

Gmeiner<sup>134</sup> undertook a similar substitution on a mesyl-protected alcohol  $\beta$  to a tertiary nitrogen [316] using  $\text{Me}_2\text{CuLi}$ ,  $\text{Bu}_2\text{CuLi}$  and  $\text{Ph}_2\text{CuLi}$  but published no yields. He later<sup>135</sup> reported a reaction between a lithiocuprate and the mesylate [317]. Yields of only 47-48% were reported and only with 20 equivalents of  $\text{RLi}$  and 11 equivalents of  $\text{CuI}$  at  $-20^{\circ}\text{C}$  for 16 to 23 hours. Gmeiner *et al* gave no explanation for the sluggish nature of these reactions.



[316]

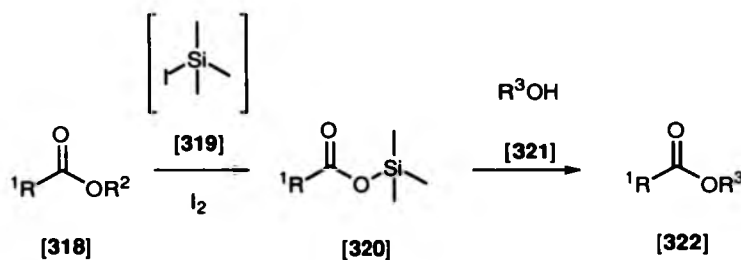


[317]

### 3.3.4 Results and Discussion. Synthesis of indolizidine analogues by transesterification.

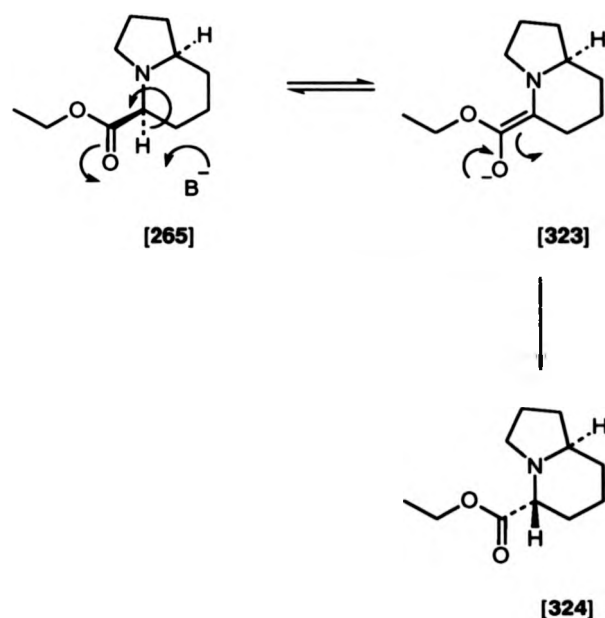
Normal transesterification or alkoxy-de-alkoxylation can be catalysed by acids or bases, usually at high temperature.<sup>136</sup> If acid or base-sensitive moieties are present in the molecule, transesterification can be undertaken at neutral pH, under mild conditions, for example by the use of iodotrimethylsilane, with catalytic amounts of iodine (Scheme 58).<sup>137</sup> The ester is first O-silylated then the

trimethyl silyloxy group is displaced by the alcohol [321] (which is the solvent in many cases).



Scheme 58

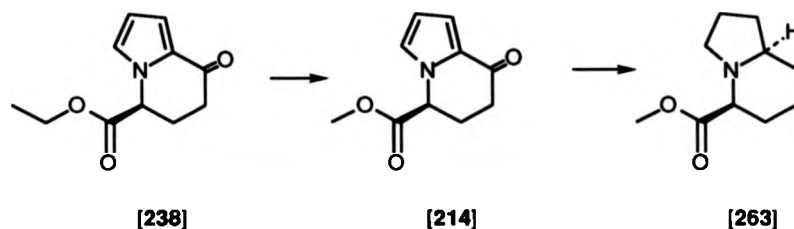
To avoid complications resulting from the hydroxyl functionality in building block [264] the transesterification studies were first carried out on the indolizidine [265]. Transesterification can be either acid- or base-catalysed. An acid-catalysed method would avoid base-catalysed epimerization at C(5) *via* the enolate [323] (Scheme 59).



Scheme 59

The acid-catalysed transesterification of [265] produced only base line material. Therefore the reaction was attempted with the pyrrole [238]. If the ethyl group was replaced with a sterically more demanding group, the hydrogenation would proceed in a more diastereofacial fashion. To this end the ethyl ester  $\alpha$ -ketopyrrole [238] was dissolved in isopropanol with catalytic quantities of concentrated HCl. No reaction occurred. A less sterically demanding alcohol was also used to test the reaction. Acid-catalysed methanolysis proceeded smoothly giving, after hydrogenation, 5-methoxycarbonylindolizidine [263](Scheme 60) with an  $[\alpha]_D$  comparable to that produced by our more direct route. This indicated that the transesterification had proceeded with retention of configuration at C(5).

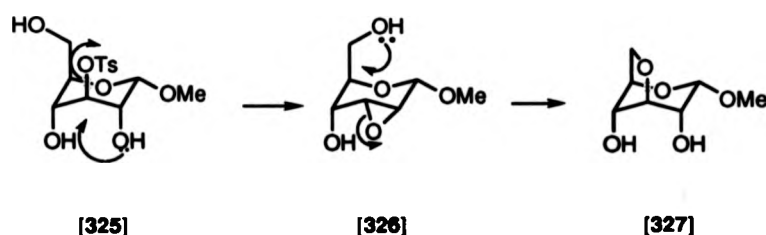




Scheme 60

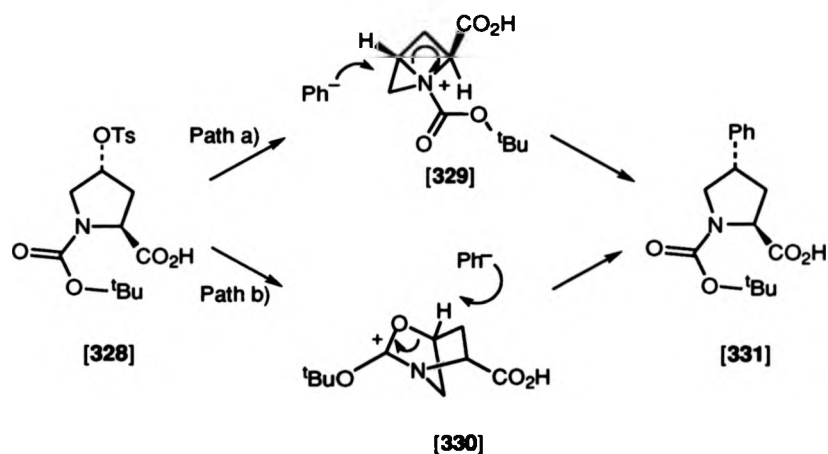
### 3.4 Discussion : the effect of $\beta$ -amino groups on tosylation reactions.

To rationalise the low yields in the tosylation reactions, Hayworth's concept of ring conformation and 'neighbouring group effects' was considered.<sup>138</sup> Isbell rationalised the formation of the 3,6-anhydro- $\beta$ -methyl glucoside [327] from [325], originally reported by Peat and Wiggins,<sup>139</sup> by two successive  $S_N2$  reactions, the first involving a tosylate displacement producing the epoxide [326], the second generating [327] (Scheme 61).



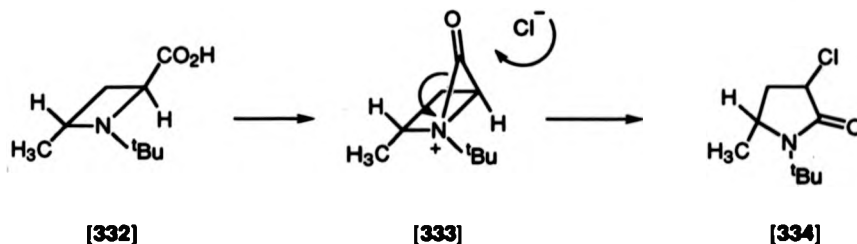
Scheme 61

Evidence for an intermediate aziridinium ion was presented by Tottathil *et al*<sup>140</sup> in the reaction of phenyl lithiocuprate with the pyrrolidine [328]. They found that the stereochemistry at C(4) was retained whereas in an  $S_N2$  reaction, inversion would be expected. Their data indicated that the reaction was in fact two consecutive  $S_N2$  reactions, the first step being an internal displacement of the tosylate group (Scheme 62).



Scheme 62

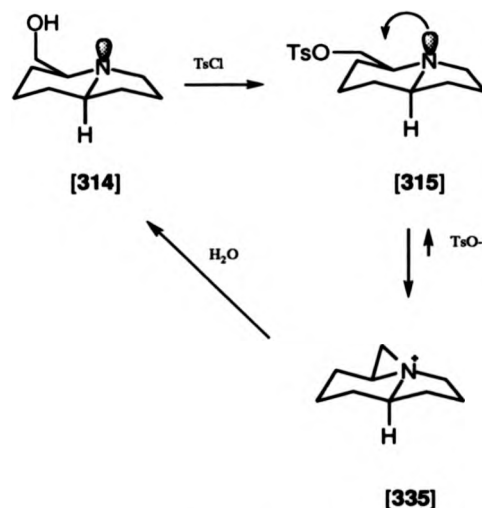
They reasoned that the reaction could proceed *via* two separate pathways. The first, path a), proceeds *via* the aziridinium ion [329] in analogy to Wasserman's bicyclic aziridinone ion [333] (Scheme 63).<sup>141</sup> Attack of the carbanion on [329] yielded the pyrrolidine [331]. In path b) the mechanism is thought to proceed *via* the bridged oxazinium ion [330] then on to the pyrrolidine [331]. Intra-molecular displacement by the lone pair on nitrogen to afford an aziridinium ion has also been postulated in the chemistry of castanospermine derivatives.<sup>142</sup>



Scheme 63

Based on these ideas, an intermediate aziridinium ion [335] as the displacement product of the tosylate group may be considered. This can be in

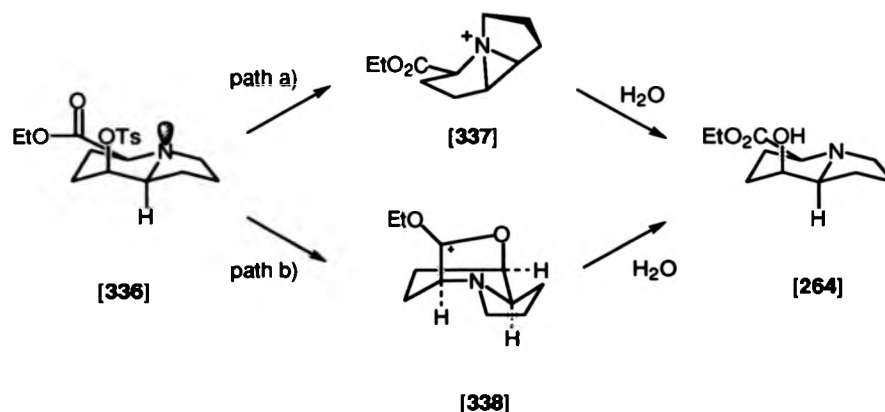
equilibrium with the tosylate [315]. Aqueous work up would yield the starting material [314] by attack of water on [335] (Scheme 64).



Scheme 64

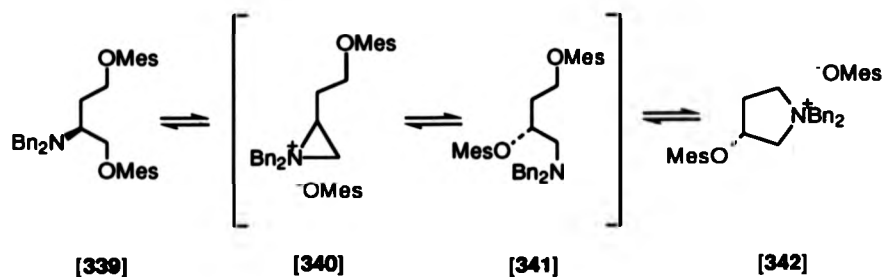
In the case of the 5,8-disubstituted indolizidine [264] there are, as in Tottathil's pyrrolidine [328], two possible displacement products, the aziridinium ion [337] from attack of the nitrogen lone pair displacing the tosylate of [336] (path a) and the bridged indolizidinium ion [338] from participation of the ester group of [336] (path b) (Scheme 65).

If simple models are made and the trajectory of attack considered (the concept of 'back-side attack') then these intermediates are not plausible in the case of the ester [336], though the ideas are valid with respect to the alcohol [314].



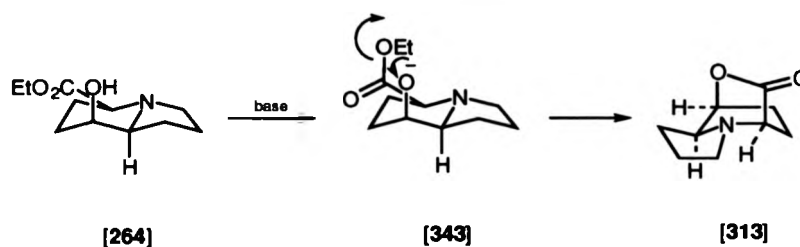
Scheme 65

A recent paper by Gmeiner *et al*<sup>135</sup> found that a mesyl group  $\beta$  to the protected tertiary nitrogen in [339] was unstable. It could be 'characterised by immediately performed  $^1\text{H}$  NMR spectroscopy' but soon rearranged via the intermediates [340] and [341] to give, after purification, the pyrrolidinium salt [342] (Scheme 66).



Scheme 66

The isolation of the tricyclic lactone [313] as a crystalline solid (Section 3.2.5) can be rationalised by the intra-molecular attack of the alkoxide ion [343] on the ester functionality of [264] yielding [313] (Scheme 67).



Scheme 67

### 3.5 Summary.

A synthetic strategy has been devised to approach the naturally occurring 5-mono and 5,8-disubstituted indolizidines [212] and [213] proceeding through the tosylates [315] and [308] respectively. In both these intermediates, the tosylate groups are  $\beta$  to the tertiary nitrogen. The synthetic problems which arose, *viz.* the low yields in the activation of alcohols as tosylates and the failure of nucleophilic substitutions at these sites both seem to be due to this  $\beta$  relationship. This suggests that alternative strategies must be developed involving only reactions in which a  $\beta$ -amino group will not interfere.

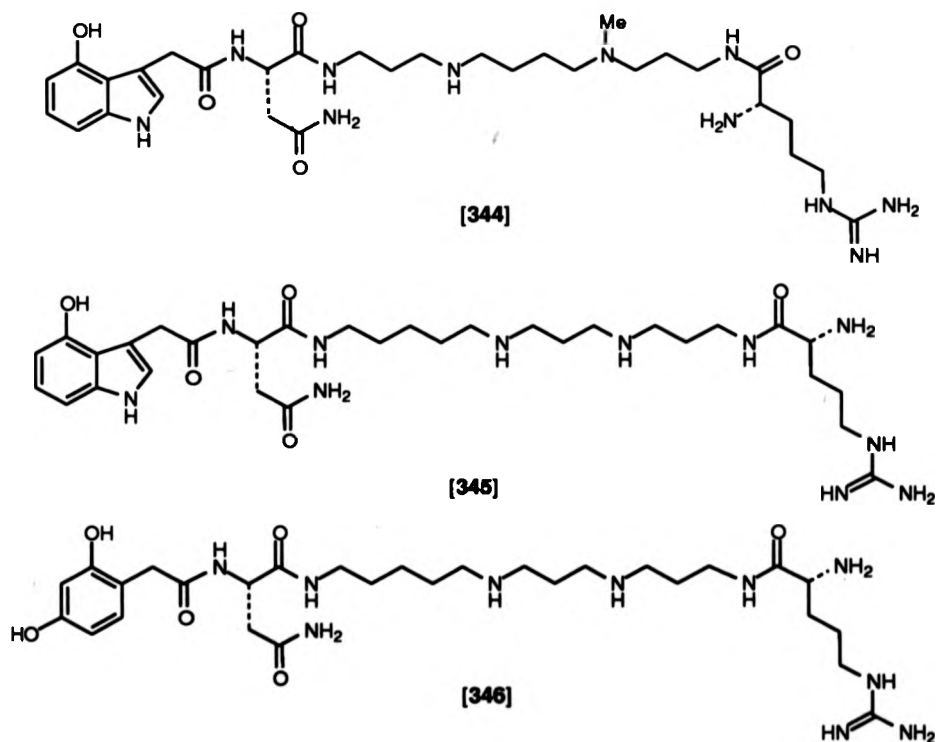
A possible alternative approach to the 5-side chain would be *via* Grignard reaction of ester [264], followed by a deoxygenation to yield compounds [212]. In summary, the ideas behind the activation/substitution approach have good literature precedent but, in our case, the  $\beta$ -nitrogen rendered it problematic.

**Spider toxins. Chapter: 4**

## 4.1 The 'Argiotoxins'.

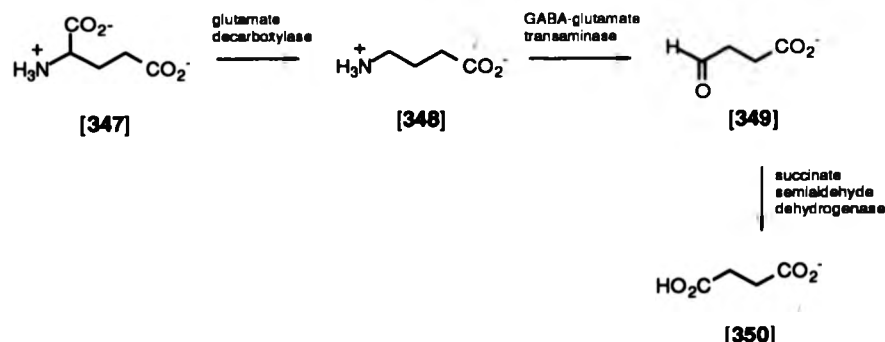
### 4.1.1 Origins.

The interruption of glutamate-mediated neuromuscular transmission in invertebrates is a strategy employed by spiders for predation. The venom of spiders of the class *Argiope* (e.g. *Argiope aurentia*, an Orb weaver spider) contains a mixture of non-peptide toxins, low molecular weight 'argiotoxins' [344](636), [345](659), [346](673), that have been found to be potent blockers of neuromuscular transmission both in insects<sup>143</sup> and mammals.<sup>144</sup>



### 4.1.2 Biological Activity.

The glutamate receptors of the central nervous system of man and other mammals are believed to be involved both in the higher neural functions, such as memory and learning, and in neurological disorders *e.g.* hypoxemia, epilepsy, Huntington's, Alzheimer's and Parkinson's diseases.<sup>145</sup> Glutamate [347], as well as controlling the movement of insect skeletal muscle, is an active neurotransmitter in the mammalian brain. However, too much glutamate can cause brain damage. In the wake of a stroke or epileptic fit, the blood supply to the brain is reduced and glutamate levels build up. This overwhelms the enzymatic degradation of glutamate *via*  $\gamma$ -amino butyrate [348](GABA), an inhibitory transmitter (Scheme 68), and the glutamate becomes 'excitotoxic', the constant excitement causing cell death.



Scheme 68

It has been suggested by Jackson<sup>146</sup> that the argiotoxin venoms are specific competitive inhibitors of the glutamate receptors, binding to them and thus denying access to the glutamate.

Neurobiological research, probing the structure and activity of sites of neurological interest, needs blockers of a high degree of specificity as pharmacological probes. Due to their specificity, argiotoxins would seem ideal



for this purpose and could lead to structural elucidation of receptor sites and hence, perhaps, to treatments for neurological disorders.

#### 4.1.3 Neurotransmission

Neurotransmission is the process by which a nerve cell passes on a signal to another nerve cell, muscle or gland. The structure of a nerve cell or neuron is shown in Figure 14.

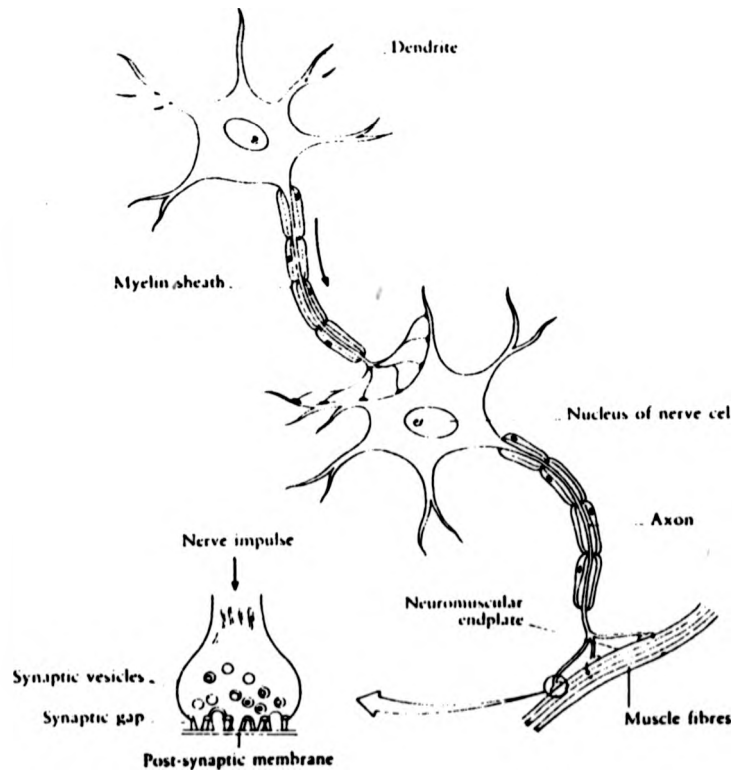


Figure 14. Structure of a nerve cell.<sup>147</sup>

The neuron consists of a cell body, branched into dendrites for connection to other cells, and an axon. In man, the axon can vary in length from less than 1

mm in the brain to almost 0.5 m in the spinal column (a factor of  $10^6$  longer than the diameter of the cell body). The axon is coated in a layer of lipid called the myelin sheath which acts as an electrical insulator. The difference between nerve cells and other cell types is that nerve cells are excitable.

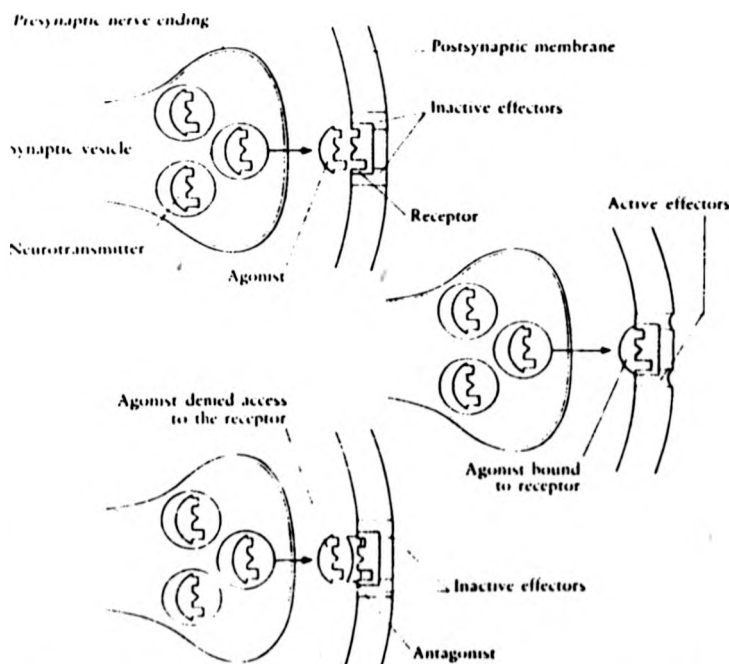
All cells have a resting potential, a potential difference brought about by a difference in the concentration of ions inside and outside the cell. The cell membrane is selectively permeable to potassium and chloride ions, being 50-100 times less permeable to sodium ions. All these ions move across the cell membrane through specific ion channels which span it. Excitation of the nerve cell is brought about by local changes in the concentration of ions causing  $\text{Na}^+$  to enter the cell. This leads to a depolarisation and a subsequent expulsion of  $\text{K}^+$  from the cell to attain the original resting potential. To attain the original state in terms of ion concentration,  $\text{Na}^+$  ions must be pumped from the cell (with concomitant entry of  $\text{K}^+$  ions to balance the charges).

The myelin sheath is an insulating layer but is not continuous, as indicated in Figure 14. The sodium and potassium channels are therefore only active in the gaps. This leads to an electrical impulse moving along the nerve from gap to gap as a wave of polarisation/depolarisation.

There is not a single nerve in the body long enough to connect the brain to, for example, the big toe so the message must be passed from neuron to neuron. There is no direct connection between neurons or between neurons and muscles/glands. At the junction between these, the electrical signal is converted into a chemical one in the form of a neurotransmitter which diffuses across the gap or 'synapse'. The neurotransmitter is released pre-synaptically and fits into a post-synaptic receptor, a glycoprotein of specific 3-dimensional structure. The neurotransmitter, due to its shape, fits into the receptor, triggering a new

electrical impulse which moves down the next neuron and so on. The bound neurotransmitter is then either destroyed enzymatically or absorbed for future use.

Drugs and other xenobiotics that interfere with neurotransmission do so as either 'agonists' or 'antagonists' (Figure 15). Those that elicit a response are termed agonists, whereas those that bind to the receptor, thus denying the neurotransmitter access but eliciting no response, are termed antagonists.



**Figure 15. Xenobiotics as Agonists and Antagonists.**<sup>147</sup>

Argiotoxins are glutamate antagonists. They bind to the glutamate receptor and deny the glutamate access. There are three major types of glutamate receptors:

- N-methyl D-aspartate (NMDA);
- quisqualate (AMPA);
- kainate.

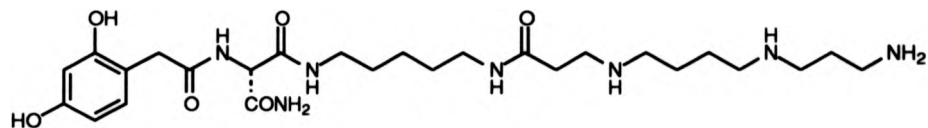
These classifications are based upon the receptor's response to agonists. Over the last few years, many NMDA antagonists have become available.<sup>148</sup> Sources of antagonists for quisqualate and kainate receptors seem to be the venoms evolved by predators of insects, whose neurotransmission is mediated by quisqualate-type receptors.

Argiotoxins have an internal polyamine chain flanked on one side by an *N*-terminal arginine and on the other by a hydroxyarylacetamide containing terminal asparagine. This arrangement of amino acids linked *via* a central polyamine is shown in Figure 16.

**Figure 16. General structure of an Argiotoxin.**

#### 4.2.1 JSTX nephilatoxins.

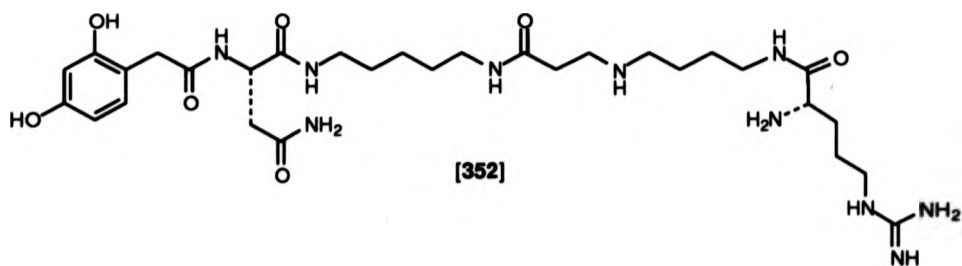
There is only one naturally occurring JSTX, viz. [351] JSTX-3. Analogues 2 and 4 have been synthesised. Hashimoto<sup>149</sup> noted the 'presence of a 2,4-dihydroxyphenylacetyl-aspariginyl cadaverine moiety'. This is elongated by a 8-amino-4-azaoctanoic acid (putreanine) fragment.



[351]

#### 4.2.2 NSTX nephilatoxins.

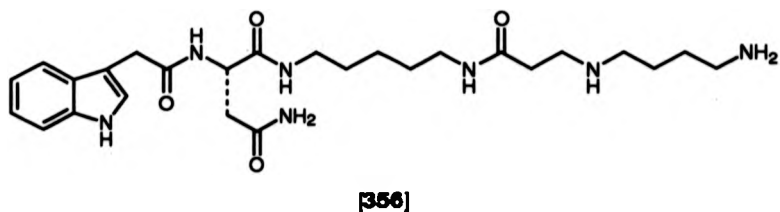
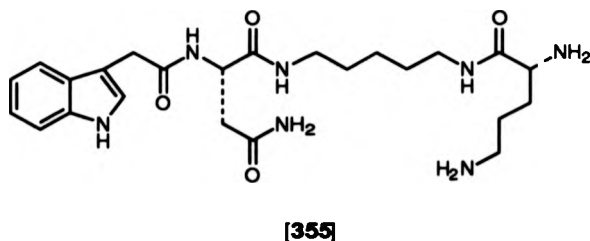
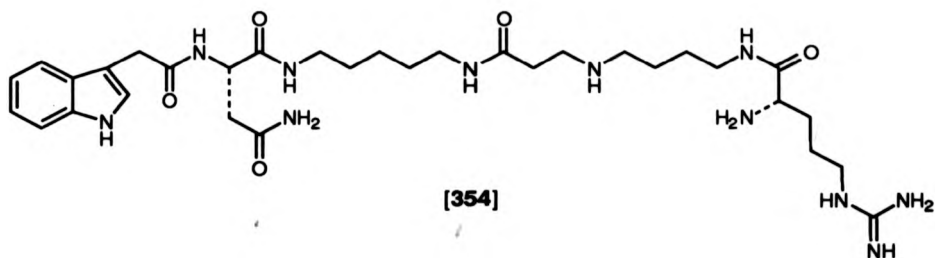
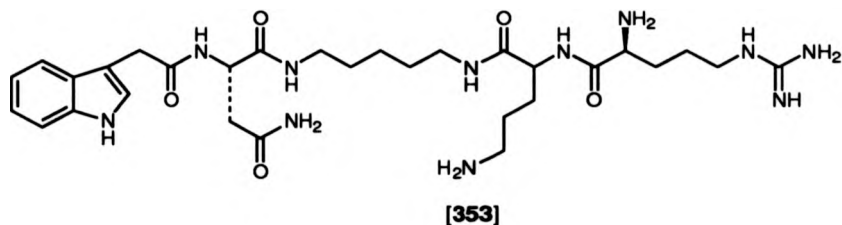
Once again there is only one naturally occurring example in the NSTX subclass, [352] NSTX-3.<sup>150</sup> This contains Hashimoto's fragment, the putreanine fragment and terminates in *N*-terminal arginine.



[352]

#### 4.2.3 NPTX nephilatoxins.

Here there are four examples,<sup>151</sup> [353]-[356] bearing, at one end of the polyamine function an indole-3-acetyl containing terminal asparagine, the other end terminating either as the polyamine, or with *N*-terminal arginine.



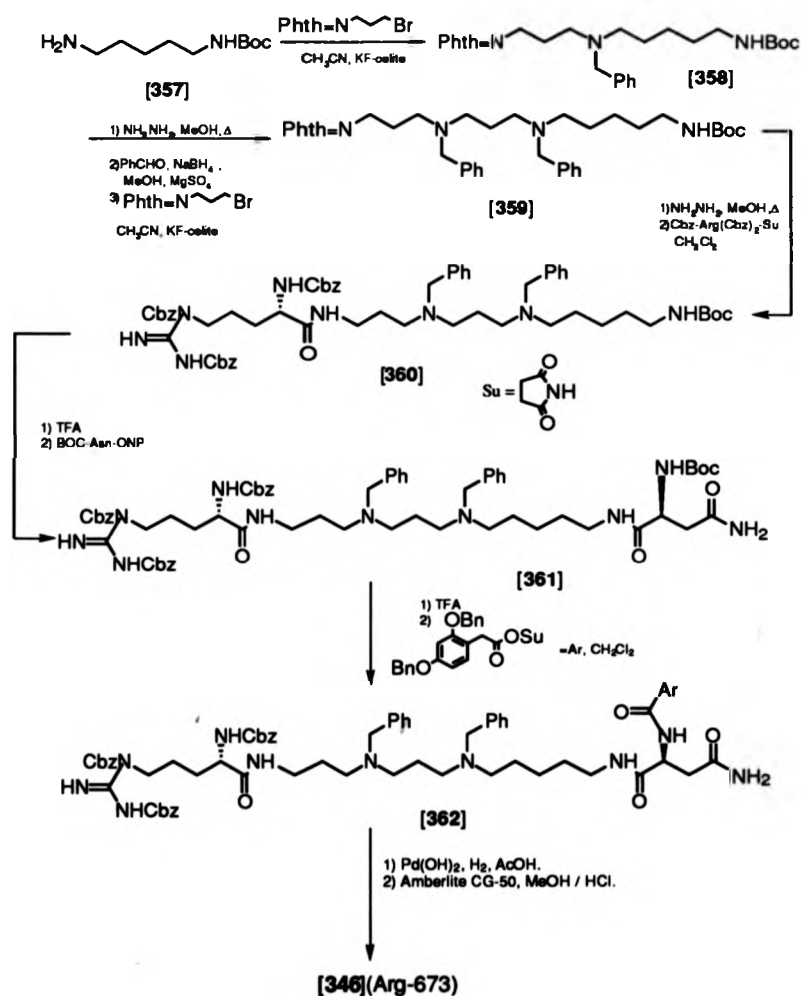
In summary, the basic components of these classes of *arachnid* venoms are:

- an arylacetamide C-terminal group;
- a linking polyamine of varying structure;
- a terminal arginine (sometimes).

### 4.3 Chemical Syntheses.

Spider toxins of argiotoxin classes contain a variety of highly polar functional groups, aryl hydroxyl groups, numerous amines (primary, secondary, tertiary), and guanidine residues. All the syntheses to date make extensive use of standard protection/deprotection procedures, using the benzyloxycarbonyl (Cbz), *t*butyloxycarbonyl (Boc) and benzyl (Bn) groups (Scheme 69).<sup>152</sup>

Although these protection-deprotection techniques are well documented and, in the main, high yielding, two steps are added to the synthesis for each protecting group used. In addition, use of some of these protecting groups are cost prohibitive on a commercial scale. There are also safety concerns with, for example, the use of the use of TFA. It would clearly be preferable to eliminate the need for protecting groups. What is therefore required is a system which can distinguish between the various, similar, functional groups. Enzymes achieve this by binding molecules in a highly specific manner. They place only one of the required groups into a catalytic environment, thus greatly accelerating reaction at that particular position.



Scheme 69

## 4.4 Enzymatic syntheses.

The number of steps in the synthesis of an argitoxin could thus, in principle, be reduced by the use of enzymatic means to distinguish between the various nitrogen functionalities. Another advantage of enzymatic methods of synthesis is that mild conditions are used. This is particularly important with moieties such as tryptophan, which is particularly sensitive to acid and base.



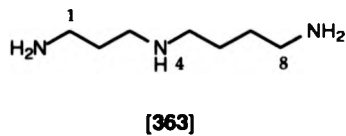
Polyamines are found in all higher organisms. They appear to be vital for regulating cell metabolism, though their exact function remains unknown.<sup>153</sup> Polyamines are polycationic at physiological pH and are closely associated with DNA in the nuclei of cells. Cells undergoing rapid turnover have been found to have elevated levels of polyamines. Polyamine metabolism in mammals is well documented.<sup>154,155</sup> It may be useful here to clarify the nomenclature and numbering system of polyamines Table 3.

| Systematic name   | Trivial name                            | Alternatives                       |
|---|---|------------------------------------|
| 1,4-Butanediamine   | Putrescine                              | 1,4-Diaminobutane                  |
| <i>N</i> -(4-Aminobutyl) acetamide                            | <i>N</i> -Acetylputrescine              | <i>N</i> -Acetyl-1,4-Diaminobutane |
| <i>N</i> -(3-aminopropyl)-1,4-butane diamine                  | Spermidine                              | -                                  |
| <i>N</i> -[4-[(3-aminopropyl)-amino]butyl] acetamide          | <i>N</i> <sup>6</sup> -Acetylspermidine | Acetylspermidine A                 |
| <i>N</i> -[3-[(4-aminobutyl)-amino]propyl] acetamide          | <i>N</i> <sup>1</sup> -Acetylspermidine | Acetylspermidine B                 |
| <i>N</i> -4-(aminobutyl)- <i>N</i> -(3-aminopropyl) acetamide | <i>N</i> <sup>4</sup> -Acetylspermidine | -                                  |

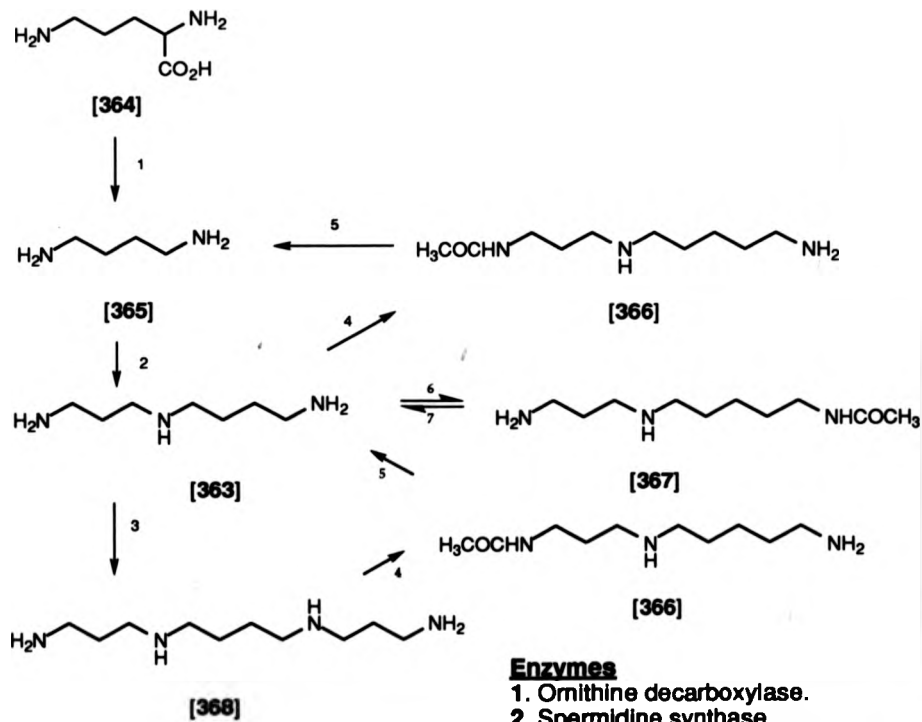
Table 3

Numerical terminology is based on a modified aza-nomenclature ( IUPAC Rule 814.6), as convention requires the secondary nitrogen is assigned the lowest

number ( i.e.  $N^4$  rather than  $N^5$  ) as in Figure 17.



**Figure 17. Numbering system of polyamines.**



## Enzymes

1. Ornithine decarboxylase.
2. Spermidine synthase.
3. Spermine synthase.
4. Spermidine/spermine synthase.
5. Polyamine oxidase.
6. Spermidine N-acetyl transferase.
7. N-Acetyl spermidine deacetylase.

### Scheme 70

As can be seen from the metabolic pathway scheme (Scheme 70),<sup>154</sup> there are two possible ways of enzymatic differentiation between  $N^1$  and  $N^8$ ;

- selective hydrolysis;
- selective acylation.

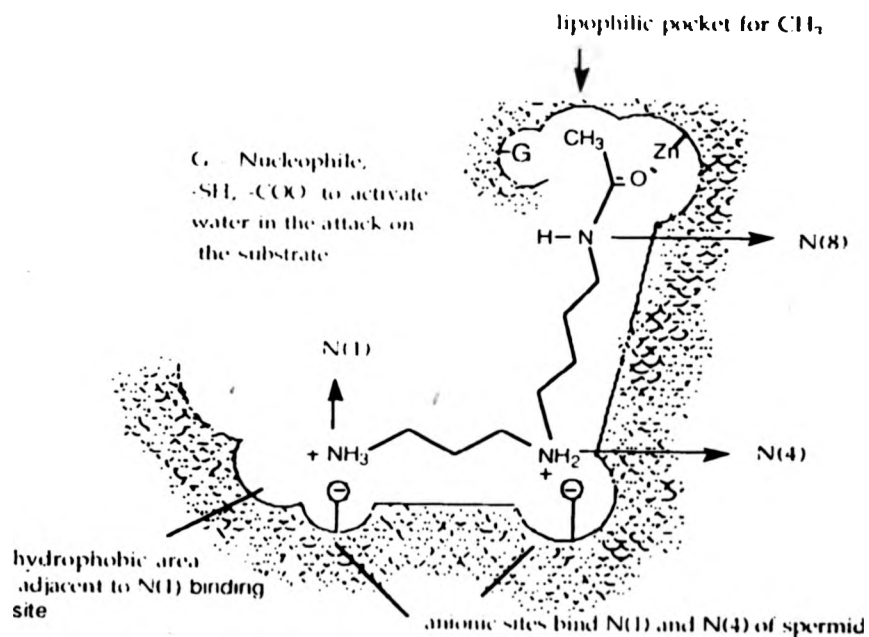
#### 4.4.1 Selective Hydrolysis.

Marchant<sup>156</sup> showed that the metabolic fates of  $N^1$ -acetylspermidine and  $N^8$ -acetylspermidine are different *in vivo*, as shown in Scheme 70.  $N^1$ -acetylspermidine undergoes oxidative cleavage by polyamine oxidase to yield putrescine whilst  $N^8$ -acetylspermidine, under the influence of  $N$ -acetylspermidine deacylase yields spermidine and acetic acid.  $N$ -Acetyl spermidine deacylase was first discovered by Libby in calf liver<sup>157</sup> and by Blankenship in rat liver.<sup>158</sup> Further work by Marchant *et al* showed that  $N^1$ -acetylspermidine did not undergo deacetylation by  $N$ -acetylspermidine deacylase (using rat liver cytosol fraction) whilst  $N^8$ -acetylspermidine did. The differentiation of  $N^1$ - versus  $N^8$ -acetylspermidine would seem to be due to a highly specific active site in the  $N^1$ -acetylspermidine deacylase. Dredar *et al*, from their work on inhibitors of  $N^8$ -acetylspermidine, proposed the bonding interaction scheme for the active site shown in Figure 18.<sup>154</sup> Thus, the  $N^1$ -acetylspermidine does not fulfill the steric/electronic demands of the active site and so is not deacetylated.

#### 4.4.2 Selective Acetylation.

Cells contain cytoplasmic enzymes capable of converting spermine to spermidine and spermidine to putrescine. Both these are two-step processes *via*  $N^1$ -acetyl derivatives of spermine and spermidine respectively<sup>159,160</sup>. In addition, there is a nuclear enzyme that acetylates spermidine selectively at the

$N^8$  position. However,  $N^8$ -acetylspermidine is not seen in appreciable quantities in most tissues due to the probable action of a cytoplasmic enzyme that selectively deacetylates  $N^8$ -acetylspermidine<sup>156</sup>. It can be inferred both from these results and from the pathway proposed in Scheme 70 that it is possible to distinguish between the  $N^1$ - and  $N^8$ -acetyl groups on spermidine by the action of these rat liver cell fractions.



**Figure 18.** Active site of  $N(8)$ -spermidine deacetylase.

#### 4.4.3 Single enzyme preparations.

Cytosolic fractions of animal tissues contain a mixture of enzymes. In many cases it is more informative and practically more efficient to use commercially available single enzyme preparations. These 'solid' enzyme preparations are of greater use than the aqueous cytosolic fractions in systems where the solubility of the substrate in water is not significant. Their use in these

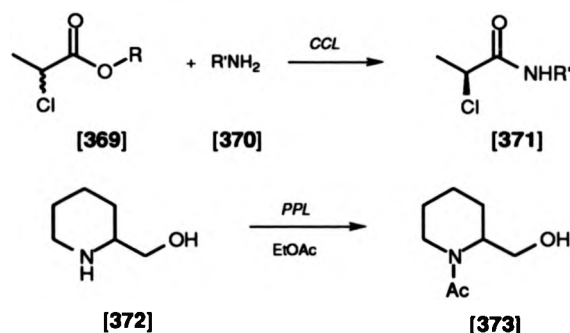
'lipophilic' systems requires a more imaginative and inventive approach to conditions, solvents *etc.*

## 4.5 Enzymes in organic solvents.

As early as 1937 Bergman and Fraenkel-Conrat<sup>161,162</sup> suggested that the reversibility of protease catalysed reactions could be utilised for the formation of peptide bonds. During the past decade such enzymatic catalysis has attracted much attention. This has led to the development of many systems that successfully attain enzymatic activity in predominantly organic media. These include the direct suspension of enzyme particles in the organic phase, covalent modifications to render the enzyme soluble in organic solvents and the use of biphasic media as well as water miscible organic solvents. All these have their advantages and disadvantages, though so far a direct comparison of enzymatic stability and activity has not been possible.

### 4.5.1 Anhydrous organic solvents.

The use of lipases and proteases in essentially anhydrous solvents has received much attention.<sup>163,164,165</sup> The range of reactions (Scheme 71) includes: general amide synthesis<sup>166</sup> using various biocatalysts in hexane and the production of amides enantioselectively<sup>167,168</sup> and transesterification<sup>169</sup> using such media such as hexane, 1,4-dioxane, or diisopropyl ether. *N*-Acetylations, however, are rarer than *O*-acetylations and have mostly been used in the field of peptide synthesis.<sup>170</sup> *N*-Acetylation has been accomplished for primary<sup>165,171</sup> aromatic<sup>172</sup> and secondary amines<sup>173</sup> using ethyl acetate as both solvent and transfer reagent.



Scheme 71

Surprisingly, even hydrolysis can be performed in "anhydrous" diisopropyl ether.<sup>174</sup> Kanerva and Klivanov<sup>175</sup> undertook mechanistic studies using *Subtilisin carlsberg* as a model as it had been shown to have protease activity in anhydrous organic solvents. They concluded that there were "profound" mechanistic similarities in organic media and aqueous media. This suggested that the transition state in both media is essentially the same.

#### 4.5.2 Biphasic systems

Numerous groups have achieved the synthesis of peptides in two-phase reaction mixtures,<sup>176</sup> viz. an aqueous phase (containing the enzyme) and a water-immiscible organic phase. The equilibrium is shifted due to the preferential solubility of the peptide formed in the organic phase with respect to the starting material. The main limitation of the biphasic system seems to be biocatalyst stability, which often results in the precipitation of the enzyme as a gel. Halling showed that biocatalyst stability increased as the polarity of the solvent decreased.<sup>177</sup>

Many groups have investigated the use of water miscible co-solvents<sup>178,179</sup> and the changes they induced in the catalytic activity of  $\alpha$ -

chymotrypsin and laccase. The best co-solvents were found to be the polyalcohols ethylene glycol and glycerol (in which  $\alpha$ -chymotrypsin inactivation is reversible). The reasons proposed for the changes in catalytic activity of the biocatalysts<sup>178,180,181</sup> in these systems are summarised below:

- some organic co-solvents (and water) may act as substrates for, and thus competitive inhibitors of enzyme activity;
- the co-solvent may act as a specific inhibitor;
- addition of a co-solvent changes the bulk properties of the solution, thus changing the interactions between, enzyme, substrate and solution;
- the co-solvent may denature the enzyme.

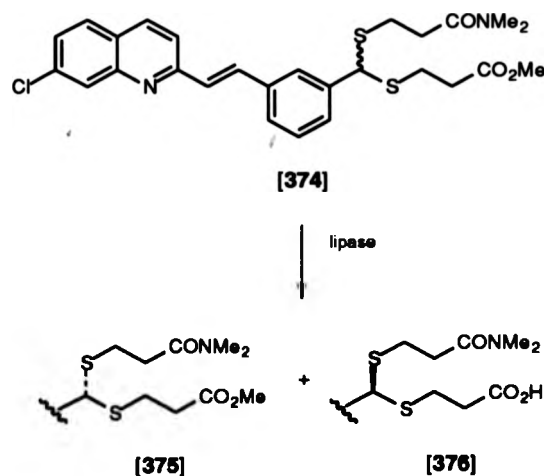
Mozhaev *et al* produced a quantitative correlation of denaturing ability *vs.* hydrophobicity.<sup>178</sup> This points to the general rule that the best co-solvents are the most hydrophilic ones. The authors add a cautionary note that this relationship can only be applied to organic solvents with the same homological group.

Enzymes have been covalently modified by the attachment of polyethylene glycol residues.<sup>182</sup> This rendered the enzyme soluble in benzene both for peptide formation and hydrolysis. This was found to be quite satisfactory, the catalytic activity of the enzymes being only three to four fold lower than in aqueous buffer. Reasonable evidence was found that the enzyme structure is more compact in water restricted environments, limiting conformational mobility. Another interesting discovery was that the minimum amount of water required for enzymatic activity in anhydrous organic media was *ca* 0.04%, the optimum being 0.09% (by volume).<sup>183</sup> Enzymes in 'anhydrous' systems, require a minimum amount of water to maintain their shape and remain active. The conformation of an enzyme is maintained by hydrogen bonds and salt bridges. All organic solvents will allow a certain amount of water to dissolve

in them. If this is not present then the water maintaining the shape of the enzyme will dissolve in the solvent and the enzyme will be denatured and thus deactivated.

#### 4.5.3 Emulsions.

The final way to overcome poor solubility of organic reagents in aqueous systems or to maintain favourable equilibrium changes is to use oil-in-water emulsions containing for example, non-ionic surfactants. Hughes *et al*,<sup>184</sup> in the enzymatic hydrolysis of dithioacetal esters (Scheme 72) using lipase from *Pseudomonas sp.*, found hydrolysis occurred very slowly.



Scheme 72

This was attributed to the presence of a liquid-solid interface produced by the insolubility of the esters, so Triton X-100 was used to aid their solubility. It was found that the addition of water-miscible solvents such as DMF, ethanol and 2-propanol decreased the already sluggish rate of hydrolysis. So, in cases where the optimum organic co-solvent proves elusive the addition of Triton X-100 or

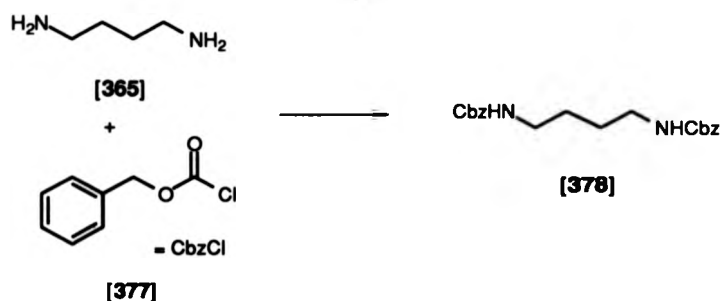


other surfactants (though X-405 and X-705 were in this case less effective) provides another approach.

In summary, the precedent of the known metabolism of polyamines may be put forward as proof that enzymes are capable of differentiating the *N*-1 and *N*-8 termini of spermidine. This should allow selective protection of one end of the polyamine, or selective deprotection of a fully protected molecule, in order to begin the construction of one of the argiotoxins.

#### 4.6 Summary of new methodology.

It was decided to start with the deprotection of a fully protected spermidine model. The model system chosen was 1,4-diaminobutane [365] and the protecting group was benzyl formate (Cbz) (Scheme 73).



Scheme 73

The intention was to selectively deprotect one of the protected amines, to derivatise this free amine and then to use catalytic hydrogenation to remove the other Cbz group. The Cbz protecting group was particularly appropriate, as it is hydrolysed by a range of enzymes including proteases, amidases and lipases which cleave different bonds in the molecule (Figure 19). In the case of lipases a decarboxylation reaction would be expected to follow to yield the free amine. The use of acetate derivatives was also considered, as acetate is one of the more

commonly used protecting groups.

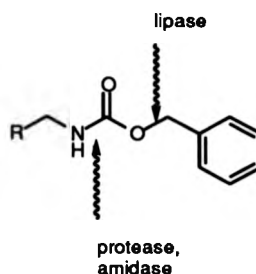
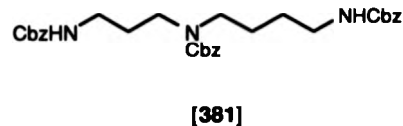
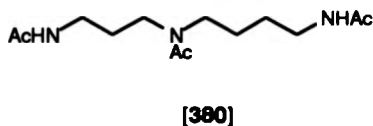
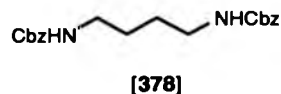
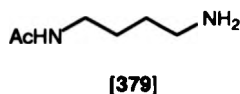


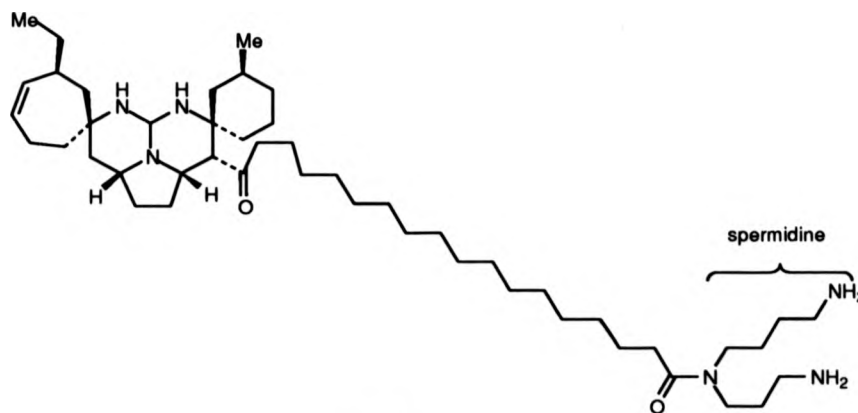
Figure 19. Enzymatic cleavage of the benzyl chloroformate protecting group

#### 4.7 Preparation of substrates.



Model compounds [379] and [378] were prepared from diaminobutane under standard conditions.<sup>185,186</sup> The structures were confirmed by  $^1\text{H}$ ,  $^{13}\text{C}$  NMR and mass spectrometry. The protected spermidine derivatives [380] and [381] were prepared by the same methods. The tris Cbz compound [381] was easily characterised by  $^1\text{H}$  and  $^{13}\text{C}$  NMR but the tris acetyl derivative [380], although apparently pure by TLC and MS, showed two sets of closely spaced resonances in both its  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. This phenomenon can be attributed to restricted rotation about the tertiary amide bond in [380]. Indeed, on warming to 337 K the signals were seen to coalesce. This feature has also been observed by Ohtani *et al* investigating the conformation of Ptilomycin A [382]

using trifluoroacetylated spermidine analogues.<sup>187</sup> The data they produced is very similar to ours and confirmed our assignments of the <sup>1</sup>H NMR spectra.



[382]

## 4.8 Results and discussion.

A variety of enzymes and solvents were employed in the attempt to deacylate bis(Cbz)-1,4-diaminobutane [378] and tris(Cbz)spermine [381]. For amidation and hydrolysis of the peptide bond a variety of enzymes may be used, not only amidases and proteases but also lipases.<sup>188,189</sup> Both the reactions proceed *via* an [enzyme-acyl] intermediate [383] and, depending on the next step, either the hydrolysis [384] or the peptide product [385] may be formed as in Scheme 74. The hydrolysis of an amide can be achieved in an aqueous system.

| Substrate | Solvent             | Enzymes  | Result      |
|-----------|---------------------|--|-------------|
| [378]     | Buffer              | $\alpha$ , $\beta$ - chymotrypsin                    | no reaction |
| [378]     | 10% DMF             | P6, CCL,<br>$\alpha$ , $\beta$ - chymotrypsin        | no reaction |
| [378]     | 10% acetonitrile    | P6, CCL,<br>$\alpha$ , $\beta$ - chymotrypsin        | no reaction |
| [378]     | 10% ethylene glycol | P6, CCL,<br>$\alpha$ , $\beta$ - chymotrypsin        | no reaction |
| [378]     | dichloromethane     | P6, CCL, PN, PS<br>$\alpha$ , $\beta$ - chymotrypsin | no reaction |
| [378]     | hexane              | P6, CCL,<br>$\alpha$ , $\beta$ - chymotrypsin        | no reaction |
| [378]     | Buffer/Triton X-100 | P6, CCL,<br>$\alpha$ , $\beta$ - chymotrypsin        | no reaction |
| [381]     | Buffer              | P6, CCL,<br>$\alpha$ , $\beta$ - chymotrypsin        | no reaction |
| [381]     | 10% DMF             | P6, CCL,<br>$\alpha$ , $\beta$ - chymotrypsin        | no reaction |
| [381]     | 10% ethylene glycol | P6, CCL,<br>$\alpha$ , $\beta$ - chymotrypsin        | no reaction |

**Table 4. Attempted hydrolysis of Cbz protected polyamines.**

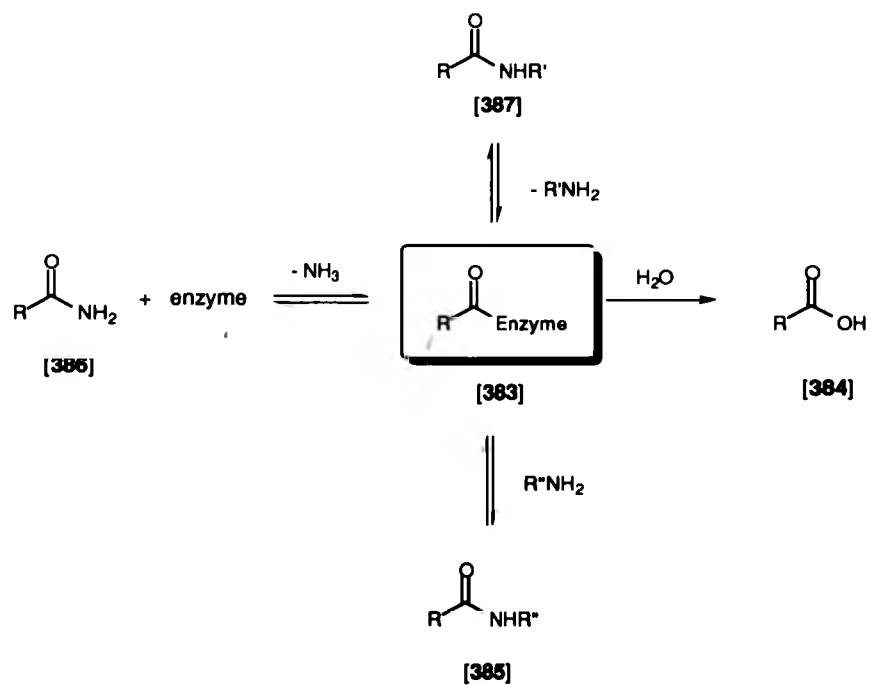
Buffer = Tris HCl, pH 7.8

P6 = Amano Protease '6'

PN = Amano Protease 'N'

PS = Amano Protease 'S'

CCL = *Candida cylindracea*. lipase



Scheme 74. Enzyme-acyl intermediate.

| Substrate | Solvent         | Enzymes   | Reaction    |
|-----------|-----------------|-----------|-------------|
| [380]     | Tris HCl Buffer | P6        | No reaction |
| [380]     | Tris HCl Buffer | PS        | No reaction |
| [380]     | Tris HCl Buffer | PN        | No reaction |
| [380]     | Tris HCl Buffer | CCL       | No reaction |
| [380]     | Tris HCl Buffer | Pronase   | No reaction |
| [380]     | Tris HCl Buffer | Acylase 1 | No reaction |
| [380]     | Tris HCl Buffer | PPL       | No reaction |

**Table 5. Attempted hydrolysis of acetate protected polyamines**

P6 = Amano Protease '6'

PN = Amano Protease 'N'

PS = Amano Protease 'S'

CCL = *Candida cylindracea*. lipase

PPL = pig pancreatic lipase.

Many different solvents have been used successfully with enzymatic activity being retained. The solvents chosen were selected by following literature precedents. From Tables 4 and 5 it can be seen that the organic solvents used were dimethyl formamide, acetonitrile and ethylene glycol (as water soluble solvents) and that hexane and dichloromethane were used in the reactions requiring biphasic or virtually anhydrous conditions. Schneider *et al* reported at the '1993 Biotransformations Update', the effect of solvents on lipase activity (Figure 20), indicating that in hydrophobic solvents such as hexane and toluene the enzyme retains almost all its activity with respect to an aqueous system.<sup>190</sup> This was also reported earlier by Klibanov<sup>191</sup> who rationalised this observation by proposing that the more hydrophilic solvents 'strip' essential water (see later) from the enzyme molecule thus diminishing enzymatic activity.

Though this data is for lipase activity, the presence of the common acyl-enzyme intermediate allows us to propose this will be the case for other classes of enzyme.

In water reduced systems (dichloromethane, hexane) the enzymes were lyophilised prior to use. This involves the dissolution of the enzyme in an appropriate pH buffer (in our case pH 7.8) and subsequent removal of the water by freeze-drying. This satisfies two of the three criteria for enzymatic activity in organic solvents.<sup>191</sup>

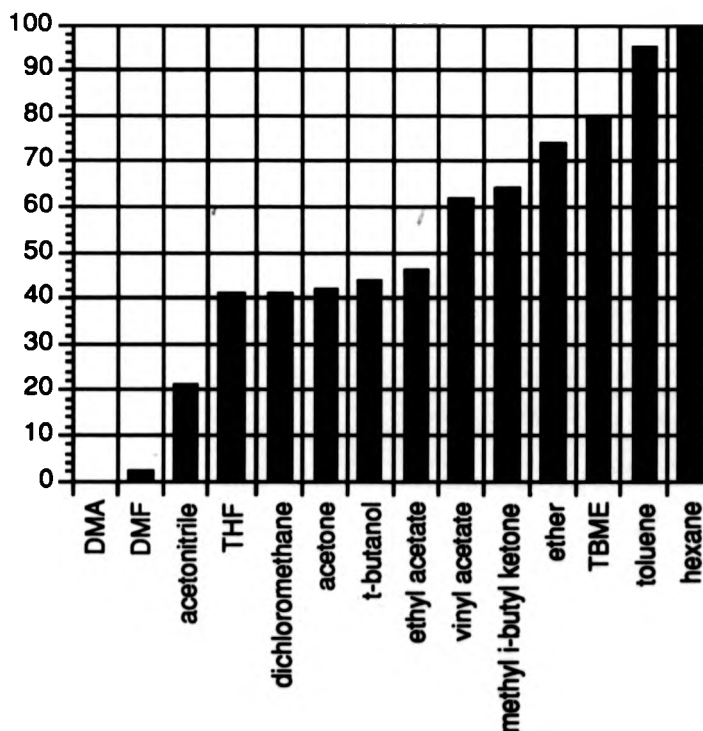


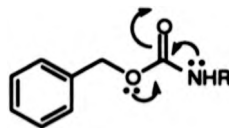
Figure 20. The effect of solvents on lipase activity.

Freeze-drying maintains a mono-layer of water molecules over the enzyme, thus the hydrogen-bonds and other forces that maintain ternary structure in the

enzyme remain, thus defining the shape and subsequent bioactivity of the active site. The second criterion, is that of 'pH memory' of the enzyme. The enzyme 'remembers' the pH of the last water it came into contact with through the charged groups at the active site. These are maintained at the pH of the buffering solution. This will affect (again) the overall shape of the active site and also the 'fit' of charge sensitive groups in the substrate. The third criterion, that of the enzyme 'suspension' being composed of as small as possible particles to increase the surface contact of the enzyme and substrate solution was fulfilled by vigorous stirring. Samples were taken directly from the stirred suspension, partitioned between ethyl acetate and water and both solutions applied to the TLC plate for analysis.

Lypholysed enzyme was also used for the buffered aqueous solutions and those that contained co-solvents for direct comparison and they were also vigorously stirred. Samples were taken, as above, from the stirred solution, partitioned between ethyl acetate and water and both organic and aqueous layers analysed by TLC.

As can be seen in Table 4, the benzyloxycarbonyl protecting group seems to be resistant to enzymatic cleavage under the many conditions tried. Others have also found this.<sup>192</sup> This result may be rationalised by the donation of electron density to the carbonyl carbon due to the dipole effects of the groups present. This decreases the electrophilicity of the carbonyl carbon (Figure 21).



**Figure 21.** Donation of electron density to the carbonyl carbon of the benzyloxycarbonyl group.



The other possible reason for the failure to cleave the benzyloxycarbonyl group is bad fit/no fit in the active site of the enzyme involved. The lipophilic nature of the substrate means that methods using enzymes in low-water systems/biphasic systems must be used. The active site of enzymes in organic media are substantially the same as in aqueous media though it seems 'tighter' in water-restricted environments. This will have the effect of increasing the specificity of the enzyme, thus an enzyme that has a wide spectrum of substrates in aqueous media will have a substantially reduced range in organic media. Though this may be a contributing factor, others have successfully cleaved large groups enzymatically, for example the lipase catalysed cleavage of  $\beta$ -naphthols.<sup>193</sup> This led us to believe that the 'deactivation' of the carbonyl carbon is the dominant factor in the inability of the enzyme to cleave the benzyloxycarbonyl group under the conditions we tried.

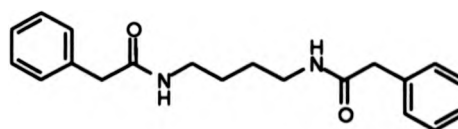
Another possible contributing factor to the lack of activity could be a stable acyl-enzyme intermediate. This would account for the TLC observation that only baseline material and starting material were seen. If there was a stable acyl-enzyme intermediate then the baseline material would be enzyme and bound starting material.

Polyamine-polynucleotide interactions are well documented,<sup>194,195,196</sup> this has even been seen for anthracyl derivatives with DNA helices.<sup>197</sup> Polyamine-protein interactions have also received attention, though in both, the exact interactions are often hard to decipher due to many non-specific interactions which tend to mask the specific ones. Polyamines have been found to be potent allosteric regulators of enzymes *e.g.* acetylcholinesterase<sup>198</sup> as well as of NMDA-type glutamate receptors.<sup>199</sup> Workers have found that though the number of charges on the polyamine were thought to be the major contributing factor to

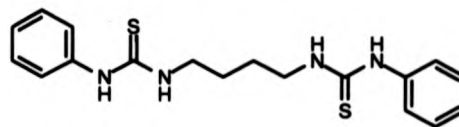
binding (spermine>spermidine>putrescine; charged groups, 4, 3, 2 respectively) there are other factors at work. Indeed  $N^1$  and  $N^8$ -acetylspermine both had a higher affinity to DNA than expected,<sup>200</sup> clearly there were non-electrostatic effects present.

To test the idea of the electron donating effect of the Cbz group being the major deactivating force on enzymatic cleavage, we decided to look at other derivatives. We began looking at the phenyl acetyl derivative [388] due to the fact that only one functional group can significantly donate electron density to the carbonyl group. We also chose the *N*-phenylaminothio carbonyl derivative [389] for investigation to determine the effect of changing nature of the heteroatoms, incorporating nitrogen and sulfur instead of oxygen.

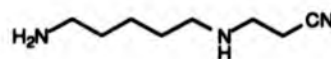
The cyano derivative [390] was prepared<sup>201</sup> with a view towards comparison to the triacetylated spermidine and the beginnings of investigations into non-electrostatic interactions to see if a stable acyl-enzyme derivative was being formed .



[388]



[389]



[390]

## 4.9 Summary.

Previous work on *in vitro* enzymatic acetylations / deacetylations had shown that some enzymes (see Scheme 70) are capable of differentiating between the  $N^1$  and  $N^8$  positions of spermidine. The proteases and lipases we chose, under our conditions (Tables 4 and 5), did not seem to have the required activity. This could be due to the electron rich nature of the carbonyl group of the Cbz protecting group, which we had chosen as the proposed deprotection method (catalytic hydrogenation) is both simple and relatively environmentally friendly.

The acetamide group should have been hydrolysed by the enzymes we used, if only nonselectively. The enzymes in Table 5 were chosen as they have been used widely in the area of biotechnology on a broad range of substrates. We thus have no explanation for the failure of these reactions.

Work on this project ceased due to the unfortunate death of Dr. D. W. Hutchinson.

## **Experimental Chapter: 5**

## 5.1 Introduction.

Melting points were determined using a Stuart Scientific SMP 1 melting point apparatus and are uncorrected. Optical rotations were recorded on an Optical Activity Ltd model AA-1000 polarimeter at 589 nm (Na D-line) with a path length of 2 dm. Concentrations (*c*) are quoted in g/100 ml. Microanalyses were performed by Mr. Paul Moore, University of Warwick on a Leeman CE440 elemental analyzer. Infra red spectra were recorded neat, in solution, or as nujol mulls, as indicated, on a Perkin-Elmer 1720X fourier transform spectrometer using sodium chloride plates. Only selected absorbances ( $\nu_{\max}$ ) are reported.  $^1\text{H}$  NMR spectra were recorded at either 250 MHz, 300 MHz, or 400 MHz on Bruker ACF 250, Varian Gemini 300 MHz, or Bruker ACP 400 instruments respectively. Chemical shifts ( $\delta_{\text{H}}$ ) are quoted in parts per million (ppm), referenced to the appropriate residual solvent peak. Coupling constants (*J*) are reported in Hertz.  $^{13}\text{C}$  NMR spectra were recorded at 63 MHz on a Bruker ACF 250 instrument unless stated otherwise. Chemical shifts ( $\delta_{\text{C}}$ ) are quoted in ppm, referenced to the appropriate solvent peak. Low resolution mass spectra and some high resolution mass spectra were recorded on a Kratos MS 80 spectrometer with only molecular ions ( $\text{M}^+$ ) and major peaks being reported with intensities quoted as percentages of the base peak. Other high resolution mass spectra were recorded on a VG Analytical ZAB-E instrument. Chemicals were purchased from Aldrich, Fluka or Sigma at the highest available grade. All solvents were purchased from Fisons Scientific Equipment at SLR grade and purified, when required, by literature methods<sup>202</sup>. Anhydrous solvents were obtained as follows;  $\text{CH}_2\text{Cl}_2$ , distilled from calcium hydride under nitrogen; THF, distilled from sodium-benzophenone under nitrogen;  $\text{C}_6\text{H}_6$ , distilled from sodium hydride under nitrogen; MeOH and EtOH, distilled from magnesium methoxide and magnesium ethoxide respectively under nitrogen. TLC was performed on

glass backed plates pre-coated with silica (0.2 mm, 60F<sub>254</sub>) which were developed using one or more of the following agents; UV fluorescence (254 nm), iodine vapour, potassium permanganate solution (0.5 % *v/v*)/Δ, sulfuric acid solution (8 % *v/v*)/Δ, ammonium molybdate (2.5 % *w/v*) and CeSO<sub>4</sub> (1.0 % *w/v*)/Δ or ninhydrin/Δ. Flash chromatography was performed on silica gel (Merck Kieselgel 60F<sub>254</sub>, 230-400 mesh). Petroleum ether used in flash chromatography was of the boiling range 40-60°C unless otherwise stated. The drying reagent used was MgSO<sub>4</sub> unless otherwise stated.

## 5.2 Experimental details for Chapter 2.

**2-Pyrrol-1-ylpentanedioic acid [225].** To a solution of (L)-glutamic acid [211] (10.16g,  $6.9 \times 10^{-2}$  moles) in glacial acetic acid (250 ml) containing sodium acetate (17.5g) at 85°C was added 2,5-dimethoxytetrahydrofuran [223] (9 ml,  $8.28 \times 10^{-3}$  moles, 1.2 equiv) dropwise. Stirring was continued for a further 10 min and the deep red solution was cooled to room temperature and poured into water (300 ml). Diethyl ether (400 ml) was then added, the aqueous layer separated and extracted with diethyl ether (400 ml). The combined organic layers were then evaporated *in vacuo* to yield a brown residue. Dichloromethane (300 ml) was added, precipitating out a brown solid which was filtered off. The remaining yellow solution was then dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield [225] as a yellow oil (3.38g 24.8%);  $[\alpha]_D^{20}$  -42.9° (c 3.0, MeOH);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3416(m), 1578(s), 1550(m);  $\delta^1\text{H}$  (250 MHz; D<sub>2</sub>O) 6.79(2H, m,  $\alpha$ -pyrrole protons), 6.14(2H, m,  $\beta$ -pyrrole protons), 4.46(1H, dd, *J*=10.0, 9.3 Hz, C(5)H), 2.32-1.98(4H, m, 2  $\times$  CH<sub>2</sub>)  $\delta^1\text{C}$  (CDCl<sub>3</sub>) 177.91, 177.46, 175.93, 61.73, 30.91, 27.10, 21.21; *m/e* (NH<sub>3</sub>, Cl<sup>+</sup>) 198(MH<sup>+</sup>)(100%).

**2-Pyrrol-1-ylpentanedioic acid dimethyl ester [224] (Method A).** To a solution of (L)-glutamic acid dimethyl ester hydrochloride [222] (8.62g, 0.04 moles) in

glacial acetic acid (250 ml) containing sodium acetate (20.22g) at 85°C was added 2,5-dimethoxytetrahydrofuran [223] (5.2 ml, 0.048 moles, 1.2 equiv) dropwise. Stirring was continued for a further 30 min and the deep red solution was cooled to room temperature and poured into water (300 ml). Dichloromethane (200 ml) was then added, the aqueous layer was separated and extracted with dichloromethane (2 x 100 ml). The combined organic layers were then dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield a red oil which was purified by Kugelrohr distillation to yield [224] as a colourless oil (4.34g 48%); (Found: C, 58.74; H, 6.64; N, 6.09%, C<sub>11</sub>H<sub>15</sub>NO<sub>4</sub> requires C, 58.66; H, 6.71; N, 6.22%);  $[\alpha]_D^{20}$  -28.6° (c 1.32, MeOH);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  1735(s);  $\delta_{\text{H}}$  (250 MHz, CDCl<sub>3</sub>) 6.70(2H, t, J=2.2 Hz,  $\alpha$ -pyrrole protons), 6.17(2H, t, J=2.2 Hz,  $\beta$ -pyrrole protons), 4.80(1H, dd, J=10.0, 9.3 Hz, C(5)H), 3.72(3H, s, CO<sub>2</sub>Me), 3.65(3H, s, CO<sub>2</sub>Me), 2.50-2.12(4H, m, 2 x CH<sub>2</sub>);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 172.51, 170.54, 119.89, 108.62, 60.24, 52.20, 51.35, 29.32, 27.83; m/e (NH<sub>3</sub>, CI<sup>+</sup>) 226(MH<sup>+</sup>, 100%).

**2-Pyrrol-1-ylpentanedioic acid dimethyl ester [224] (Method B).** To a solution of (L)-glutamic acid dimethyl ester hydrochloride [222] (1.64g, 7.7 x 10<sup>-3</sup> moles) in water (80 ml) at 70°C was added 2,5-dimethoxytetrahydrofuran [223] (1.2 ml, 9.26 x 10<sup>-3</sup> moles, 1.2 equiv) dropwise. Stirring was continued for a further 30 min and the deep red solution was cooled to room temperature. Dichloromethane (100 ml) was then added, the aqueous layer was separated and extracted with dichloromethane (2 x 50 ml). The combined organic layers were then washed with dilute HCl (200 ml), water (200 ml) and brine (100 ml), dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield a red oil, which was purified by Kugelrohr distillation (130°C, 0.2 mm Hg) to yield [224] as a clear oil (1.36 g, 70%);  $[\alpha]_D^{20}$  -33.5° (c 1.70, MeOH). Other spectral features were identical to [224] produced by method A.

**2-Pyrrol-1-ylpentanedioic acid diethyl ester [227] (Method A).** To a solution of (L)-glutamic acid diethyl ester hydrochloride [226] (8.62g, 0.04 moles) in glacial

acetic acid (250 ml) containing sodium acetate (20.22g) at 85°C was added 2,5-dimethoxytetrahydrofuran [223] (5.2 ml, 0.048 moles, 1.2 equiv) dropwise. Stirring was continued for a further 30 min and the deep red solution was cooled to room temperature and poured into water (300 ml). Dichloromethane (200 ml) was then added, the aqueous layer separated and extracted with dichloromethane (2 x 100 ml). The combined organic layers were then dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield a red oil which was purified by Kugelrohr distillation to yield [227] as a colourless oil (8.43g, 83%). (Found C, 61.85; H, 7.51; N, 5.53%; C<sub>13</sub>H<sub>19</sub>NO<sub>4</sub> requires C, 61.66; H, 7.51; N, 5.53%;  $[\alpha]_D^{20}$  -16.6° (c 1.06, EtOH);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  1738(s);  $\delta^1\text{H}$  (250 MHz, CDCl<sub>3</sub>) 6.71(2H, t, J=2.1 Hz,  $\alpha$ -pyrrole protons), 6.17(2H, t, J=2.0 Hz,  $\beta$ -pyrrole protons), 4.70(1H, dd, J=5.5, 9.7 Hz, C(5)H), 4.18(2H, q, J=6.9 Hz, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 4.1(2H, q, J=6.9 Hz, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.47-2.10(4H, m, 2 x CH<sub>2</sub>), 1.25(3H, t, J=6.9 Hz, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 1.24(3H, t, J=6.9 Hz, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>);  $\delta^1\text{C}$  (CDCl<sub>3</sub>) 172.25, 170.18, 119.94, 108.87, 61.55, 60.61, 60.52, 29.80, 27.92, 14.04, 13.96; m/e (NH<sub>3</sub>, CI<sup>+</sup>) 254(MH<sup>+</sup>, 60%) [Found, 254.1392(MH<sup>+</sup>), C<sub>13</sub>H<sub>20</sub>NO<sub>4</sub> requires 254.1387].

**2-Pyrrol-1-ylpentanedioic acid diethyl ester [227] (Method B).** To a solution of (L)-glutamic acid diethyl ester hydrochloride [226] (1.64g,  $7.7 \times 10^{-3}$  moles) in water (80 ml) at 70°C was added 2,5-dimethoxytetrahydrofuran [223] (1.2 ml,  $9.26 \times 10^{-3}$  moles, 1.2 equiv) dropwise. Stirring was continued for a further 30 min and the deep red solution was cooled to room temperature. Dichloromethane (100 ml) was then added, the aqueous layer was separated and extracted with dichloromethane (2 x 50 ml). The combined organic layers were then washed with dilute HCl (200 ml), water (200 ml) and brine (100 ml), dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield a red oil, which was purified by Kugelrohr distillation (130°C, 0.2 mm Hg) to yield [227] as a clear oil (1.36 g, 70%);  $[\alpha]_D^{20}$  -22.1° (c 1.12, EtOH). Other spectral features were identical to [227] produced by method A.



**2-Pyrrol-1-ylpentanedioic acid dimethyl ester [224] (Method C).** To a solution of [225] (3.25g, 0.016 moles) in dry methanol (200 ml) was added Amberlyst A15 (5g). The mixture was refluxed under nitrogen for 48 h, cooled and the resin was removed by filtration. The methanol was removed *in vacuo* to yield a brown oil (2.54g). The crude product was purified by column chromatography (EtOAc:Pet ether, 1:2, SiO<sub>2</sub>) to yield [224] as a colourless oil (1.62g, 44%);  $[\alpha]_D^{20}$  -29.9° (c 1.33, MeOH). Other spectral features were identical to [224] produced by method A.

**2-Pyrrol-1-ylpentanedioic acid diethyl ester [227] (Method C).** To a solution of [225] (3.25g, 0.016 moles) in dry ethanol (200 ml) was added Amberlyst A15 resin (5g). The mixture was refluxed under nitrogen for 48 h, cooled and the resin was removed by filtration. The methanol was removed *in vacuo* to yield a brown oil (3.32g). The crude product was purified by Kugelrohr distillation (150°C, 0.2 mm Hg) to yield [227] as a colourless oil (2.35g, 52%);  $[\alpha]_D^{20}$  -17.0° (c 1.12, EtOH). Other spectral features were identical to [227] produced by method A.

**4-Pyrrol-1-ylbutyric acid [254]<sup>203</sup> (Method A).** To a solution of 4-aminobutyric acid (15g, 0.146 moles) in acetic acid (350 ml) containing sodium acetate (75g, 0.915 moles) at 80°C with stirring was added 2,5-dimethoxytetrahydrofuran (19.2g, 0.146 moles) dropwise over a period of 5 min. After a further 15 min the reaction mixture was diluted with water (850 ml) and cooled to room temperature. The resultant aqueous mixture was extracted with dichloromethane (3 x 600 ml), the combined extracts were washed with water (2 x 800 ml), dried (MgSO<sub>4</sub>) and the solvent was removed *in vacuo* to yield crude pyrrole as a brown oil. Ether (150 ml) was added to triturate the impurities and these were removed by filtration. The solvent was removed *in*

*vacuo*. Kugelrohr distillation (150°C, 0.2 mm Hg) yielded [254] as a colourless oil (12.67g, 57%);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3047(s), 1713(s);  $\delta_{\text{H}}$  (250 MHz,  $\text{CDCl}_3$ ) 10.92(1H, br s,  $\text{CO}_2\text{H}$ ), 6.71(2H, d,  $J=2.2$  Hz,  $\alpha$ -pyrrole protons), 6.22(2H, d,  $J=2.1$  Hz,  $\beta$ -pyrrole protons), 4.00(2H, t,  $J=6.9$  Hz, C(4)H), 2.41(2H, t,  $J=6.9$  Hz, C(2)H), 2.14(2H, m, C(3)H);  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ ) 171.44, 119.97, 108.0, 43.62, 36.19, 36.11.

**6,7-Dihydro-5H-indolizidin-8-one [255] (PPA cyclization).** [254] (12.2g, 0.08 moles) was added to vigorously stirred polyphosphoric acid (100g, 85%) at 90°C over *ca* 5 min. The dark reaction mixture was stirred for a further 20 min and poured into ice cold water (600 ml). The aqueous layer was separated and extracted with diethyl ether (5 x 800 ml). The organic layers were combined and the solvent was removed *in vacuo*. The resultant dark yellow oil was taken up in dichloromethane (100 ml) and washed with aqueous sodium carbonate (100 ml), brine (100 ml), dried ( $\text{MgSO}_4$ ) and the solvent removed *in vacuo*. Kugelrohr distillation (220°C, 0.2 mm Hg) yielded [255] as a colourless oil which crystallized (6.5g, 56%). mp 36°C; (Found C, 71.32; H, 6.72; N, 10.38%;  $\text{C}_8\text{H}_9\text{NO}$  requires C, 71.1; H, 6.71; N, 10.4%);  $\nu_{\max}(\text{soln}, \text{CH}_2\text{Cl}_2)/\text{cm}^{-1}$  3053(w), 2962(w), 1659(s);  $\delta^1_{\text{H}}$  (250 MHz,  $\text{CDCl}_3$ ) 6.97(1H, m, C(3)H), 6.83(1H, m, C(1)H), 6.21(1H, m, C(2)H), 4.08(2H, t,  $J=5.7$  Hz, C(5)H), 2.54(2H, t,  $J=6.30$  Hz, C(7)H), 2.23(2H, m, C(6)H);  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ ) 187.18, 130.56, 125.83, 113.78, 110.20, 45.03, 36.04, 23.43.

**4-Pyrrol-1-ylbutyric acid methyl ester [256].** Dry HCl gas was bubbled through a solution of [254] (3.06g, 0.02 moles) in dry methanol (150 ml) for three hours. The methanol was then removed *in vacuo* to yield a brown oil (3.22g). Purification by Kugelrohr distillation (130°C, 0.2 mm Hg) yielded [256] as a clear oil (0.27g, 8%);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  1740(s);  $\delta_{\text{H}}$  (250 MHz,  $\text{CDCl}_3$ ) 6.69(2H, d,  $J=2.3$  Hz,  $\alpha$ -pyrrole protons), 6.17(2H, d,  $J=2.3$  Hz,  $\beta$ -pyrrole protons), 4.23(2H, t,  $J=6.9$  Hz, C(4)H), 3.71(3H, s,  $\text{CO}_2\text{CH}_3$ ), 2.79-2.43(4H, m, 2 x  $\text{CH}_2$ );  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ )

171.46, 120.46, 108.35, 51.84, 44.78, 36.28, 36.25. Further Kugelrohr distillation (220°C, 0.2 mm Hg) yielded [255] as a clear oil which later crystallized (1.43g, 53%). Analytical data was identical to [255] produced by PPA cyclization

**8-Oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid methyl ester [214] (TMSOTf cyclization).** To a solution of [224] (0.5g,  $2.22 \times 10^{-3}$  moles) in dichloromethane (100 ml) at -78°C, was added trimethylsilyltrifluoromethane sulfonate (2.4 ml, 0.013 moles, 6 equiv) dropwise over 20 min. The solution was maintained at -78°C for 2 h then allowed to attain room temperature and left stirring for 72 h. Triethylamine (1 ml) was added to quench the reaction and left for 30 min. The solution was then washed with dilute HCl (100 ml), saturated Na<sub>2</sub>CO<sub>3</sub> (100 ml) and water (100 ml), dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield a yellow oil. Column chromatography (1:1 EtOAc : pet ether, SiO<sub>2</sub>) yielded [214] as a pale yellow oil (0.20g, 50%);  $[\alpha]_D^{20} +13.2^\circ$  (c 1.04, CH<sub>2</sub>Cl<sub>2</sub>);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  1735(s);  $\delta_{\text{H}}$  (250 MHz, CDCl<sub>3</sub>) 6.94(1H, dd, J=1.5, 4.0 Hz, C(3)H), 6.80(1H, dd, J=1.5, 2.6 Hz, C(1)H), 6.21(1H, dd, J=2.6, 4.0 Hz, C(2)H), 4.88(1H, m, C(5)H), 3.67(3H, s, CO<sub>2</sub>CH<sub>3</sub>), 2.45(4H, m, 2 x CH<sub>2</sub>);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 185.94, 169.95, 130.40, 126.66, 114.61, 110.98, 56.47, 52.93, 33.06, 26.20; m/e (NH<sub>3</sub>, Cl<sup>+</sup>) 194(MH<sup>+</sup>) [Found, 194.372(MH<sup>+</sup>), C<sub>10</sub>H<sub>12</sub>NO<sub>3</sub> requires 194.0814].

**8-Oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid ethyl ester [238] (TMSOTf cyclization).** To a solution of [227] (0.83g,  $3.28 \times 10^{-3}$  moles) in dichloromethane (150 ml) was added trimethylsilyltrifluoromethane sulfonate (7 ml, 0.036 moles, 10 equiv) dropwise over 20 min. The solution was maintained at -78°C for 2 h then allowed to attain room temperature and left stirring for 72 h. Triethylamine (2 ml) was added to quench the reaction and this left for 30 min. The solution was then washed with dilute HCl (100 ml), saturated Na<sub>2</sub>CO<sub>3</sub> (100 ml) and water (100 ml), dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield a yellow oil. Column chromatography (Et<sub>2</sub>O,

SiO<sub>2</sub>) yielded [238] as a pale yellow oil (0.087g, 13%) (Found C, 63.2; H, 6.1; N, 6.6%; C<sub>11</sub>H<sub>13</sub>NO<sub>3</sub> requires C, 63.77; H, 6.28; N, 6.76%); [ $\alpha$ ]<sub>D</sub><sup>20</sup> +11.4° (c 1.42, CH<sub>2</sub>Cl<sub>2</sub>);  $\nu_{\max}$ (neat)/cm<sup>-1</sup> 1740(s);  $\delta_{\text{H}}$  (250 MHz, CDCl<sub>3</sub>) 7.06(1H, dd, J=1.4, 4.0 Hz, C(3)H), 6.88(1H, dd, J=1.4, 2.6 Hz, C(1)H), 6.31(1H, dd, J=2.6, 4.0 Hz, C(2)H), 4.92(1H, m, C(5)H), 4.24(2H, q, J=7 Hz, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.59-2.54(4H, m, 2 x CH<sub>2</sub>), 1.26(3H, q, J=7 Hz, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 185.99, 169.45, 130.39, 126.52, 114.56, 110.93, 62.13, 56.55, 33.04, 26.17, 14.03; m/e (NH<sub>3</sub>, CI<sup>+</sup>) 208(100), 106(29%); [Found 208.0974(MH<sup>+</sup>), C<sub>11</sub>H<sub>14</sub>NO<sub>3</sub> requires 208.329].

**8-Oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid methyl ester [214]** (BBr<sub>3</sub> cyclization). To a solution of [224] (2.97g, 0.0166 moles) in dichloromethane (200 ml) was added boron tribromide (1.6 ml, 0.0182 moles, 1.1 equiv) dropwise over 5 min. The solution was stirred for 15 min and water (15 ml) and saturated Na<sub>2</sub>CO<sub>3</sub> (50 ml) were added to quench the reaction and left for 10 min. The organic layer was then removed and the aqueous layer was extracted with dichloromethane (100 ml). The combined organic layers were then washed with water (100 ml), dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield [214] as a yellow oil. Column chromatography (Et<sub>2</sub>O, SiO<sub>2</sub>) yielded a pale yellow oil (0.26g, 80%); [ $\alpha$ ]<sub>D</sub><sup>20</sup> +22.8° (c 1.18, CH<sub>2</sub>Cl<sub>2</sub>). Other spectral features were in agreement with [214] produced by TMSOTf cyclization.

**8-Oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid ethyl ester [238]** (BBr<sub>3</sub> cyclization). To a solution of [227] (4.2g, 0.0166 moles) in dichloromethane (200 ml) was added boron tribromide (1.6 ml, 0.0182 moles, 1.1 equiv) dropwise over 5 min. The solution was stirred for 15 min and water (15 ml) and saturated Na<sub>2</sub>CO<sub>3</sub> (50 ml) added to quench the reaction and this left for 10 min. The organic layer was then removed and the aqueous layer was extracted with dichloromethane (100 ml). The combined organic layers were then washed with water (100 ml), dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield a

yellow oil. Column chromatography (Et<sub>2</sub>O, SiO<sub>2</sub>) yielded [238] as a pale yellow oil (3.06g, 89%);  $[\alpha]_D^{20} +22.0^\circ$  (c 1.23, CH<sub>2</sub>Cl<sub>2</sub>). Other spectral features were in agreement with [238] produced by TMSOTf cyclization.

**8-Oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid methyl ester [214] (HCl cyclization).** Dry HCl gas was bubbled through a solution of [224] (1.5g, 7.57 x 10<sup>-3</sup> moles) in dry methanol (150 ml) for 3 h. The methanol was removed *in vacuo* to yield a brown oil (1.78g). The crude product was purified by column chromatography (EtOAc:Pet ether, 1:2, SiO<sub>2</sub>) to yield [214] as a colourless oil (0.86g, 59%);  $[\alpha]_D^{20} +12.0^\circ$  (c 1.1, CH<sub>2</sub>Cl<sub>2</sub>). Other spectral features were in agreement with [214] produced by TMSOTf cyclization.

**8-Oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid ethyl ester [238] (HCl cyclization).** Dry HCl gas was bubbled through a solution of [227] (1.018g, 4.02 x 10<sup>-3</sup> moles) in dry ethanol (150 ml) for 3 h. The ethanol was removed *in vacuo* to yield a brown oil (2.54g). The crude product was purified by column chromatography (EtOAc:Pet ether, 1:2, SiO<sub>2</sub>) to yield [238] as a colourless oil (0.2g, 24%).  $[\alpha]_D^{20} +13.8^\circ$  (c 1.22, CH<sub>2</sub>Cl<sub>2</sub>). Other spectral features were in agreement with [238] produced by TMSOTf cyclization.

**8-Oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid methyl ester [214] (amberlyst A15 cyclization).** To a solution of [225] (1.5g, 7.6 x 10<sup>-3</sup> moles) in dry methanol (75 ml) was added Amberlyst 15 (1.25g). The A15 had previously been treated by washing with 1M HCl, and freeze-dried. The suspension was refluxed under nitrogen for 48 h, cooled and the resin was removed by filtration. The methanol was removed *in vacuo* to yield a brown oil (2.12g). The crude product was purified by column chromatography (Et<sub>2</sub>O, SiO<sub>2</sub>) to yield [214] as a colourless oil (0.69g, 47%);  $[\alpha]_D^{20} +14.2^\circ$  (c 1.2, CH<sub>2</sub>Cl<sub>2</sub>). Other

spectral features were in agreement with [214] produced by TMSOTf cyclization.

**8-Oxo-5,6,7,8-tetrahydro-indolizidine-5-carboxylic acid methyl ester [214] (amberlyst A15 cyclization).** To a solution of [224] (1.86g,  $8.2 \times 10^{-3}$  moles) in dry methanol (75 ml) was added Amberlyst 15 (1.37g). The A15 had previously been treated by washing with 1M HCl, and freeze-dried. The suspension was refluxed under nitrogen for 48 h, cooled and the resin removed by filtration. The methanol was removed *in vacuo* to yield a brown oil (2.12g). The crude product was purified by column chromatography ( $\text{Et}_2\text{O}$ ,  $\text{SiO}_2$ ) to yield [214] as a colourless oil (0.88g, 56%);  $[\alpha]_D^{20} +22.1^\circ$  (c 1.3,  $\text{CH}_2\text{Cl}_2$ ). Other spectral features were in agreement with [214] produced by TMSOTf cyclization.

**8-Oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid ethyl ester [238] (amberlyst A15 cyclization).** To a solution of [227] (1.06g,  $4.19 \times 10^{-3}$  moles) in dry ethanol (75 ml) was added Amberlyst 15 (1.37g). The A15 had previously been treated by washing with 1M HCl, water and freeze-dried. The suspension was refluxed under nitrogen for 48 h, cooled and the resin removed by filtration. The ethanol was removed *in vacuo* to yield a brown oil (2.54g). The crude product was purified by column chromatography ( $\text{Et}_2\text{O}$ ,  $\text{SiO}_2$ ) to yield [238] as a colourless oil (0.47g, 54%).  $[\alpha]_D^{20} +21.4^\circ$  (c 1.23,  $\text{CH}_2\text{Cl}_2$ ). Other spectral features were in agreement with [238] produced by TMSOTf cyclization.

**8-Oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid methyl ester [214] (PPA cyclization).** [224] (2.0g,  $8.8 \times 10^{-3}$  moles) was added to refluxing polyphosphoric acid (20g). The solution was refluxed under nitrogen for 48 h, cooled and poured into water (100 ml). Dichloromethane (100 ml) was then added, the aqueous layer was separated and extracted with dichloromethane (2 x 50 ml). The combined organic layers were then washed with dilute HCl (200

ml), water (200 ml) and brine (100 ml), dried ( $\text{MgSO}_4$ ) and evaporated *in vacuo* to yield a red oil. The crude product was purified by column chromatography ( $\text{Et}_2\text{O}$ ,  $\text{SiO}_2$ ) to yield [214] as a colourless oil (0.29g, 24%);  $[\alpha]_D^{20} +18.02^\circ$  (c 2.1,  $\text{CH}_2\text{Cl}_2$ ). Other spectral features were in agreement with [214] produced by TMSOTf cyclization.

**8-Oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid ethyl ester [238] (PPA cyclization).** [227] (1.99g,  $7.87 \times 10^{-3}$  moles) was added to refluxing polyphosphoric acid (20g). The solution was refluxed under nitrogen for 48 h, cooled and poured into water (100 ml). Dichloromethane (100 ml) was then added, the aqueous layer was separated and extracted with dichloromethane (2 x 50 ml). The combined organic layers were then washed with dilute HCl (200 ml), water (200 ml) and brine (100 ml), dried ( $\text{MgSO}_4$ ) and evaporated *in vacuo* to yield [238] as a red oil. The crude product was purified by column chromatography ( $\text{Et}_2\text{O}$ ,  $\text{SiO}_2$ ) to yield a colourless oil (0.29g, 18%);  $[\alpha]_D^{20} +12.9^\circ$  (c 1.26,  $\text{CH}_2\text{Cl}_2$ ). Other spectral features were in agreement with [238] produced by TMSOTf cyclization.

**Hydrogenation of 6,7-Dihydro-5H-indolizidin-8-one [255].** A solution of [255] (200 mg,  $1.48 \times 10^{-3}$  moles) in dry methanol (100 ml) containing  $\text{H}_2\text{SO}_4$  (5 drops), with catalyst (w/w with substrate either 5% Rh on alumina or 10% Pd on carbon) was hydrogenated at 55 psi for 18 h. The catalyst was removed with a celite pad and the solvent was removed *in vacuo*. Water (100 ml) was added to the residue and extracted with ether (2 x 100 ml). The aqueous layer was then extracted with dichloromethane (2 x 100 ml) and the combined dichloromethane fractions were dried ( $\text{MgSO}_4$ ) and evaporated *in vacuo* to yield a pale yellow oil (182 mg, 87%) which upon purification ( $\text{Al}_2\text{O}_3$ ,  $\text{EtOAc}$ ) was found to be a mixture of three products ( $\pm$ )[261a], ( $\pm$ )[261b], ( $\pm$ )[262].

Rh / Al<sub>2</sub>O<sub>3</sub>, [261](170.6mg): [262](11.4mg)(15:1); Pd/C, [261](9.6mg): [262](172.4mg)(1:18).

(±) 9(eq)-Octahydroindolizidin-8-ol<sup>204</sup> (±)[261a];  $\nu_{\max}$ (soln, CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 3528(m), 2712(w) cm<sup>-1</sup>;  $\delta_{\text{H}}$  (400 MHz, CDCl<sub>3</sub>) 3.36(1H, dq, J = 10.7, 4.4 Hz, C(8)H), 3.04(1H, dt, J = 2.2, 9.0 Hz, C(3)H<sub>eq</sub>), 2.87(1H, brs, OH), 2.13(1H, q, J = 9.0 Hz, C(3)H<sub>ax</sub>), 2.10-1.46(10 H, m, ring protons), 1.21-1.14(1H, m, C(7)H<sub>ax</sub>);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 72.90, 70.26, 54.07, 51.67, 33.72, 28.17, 24.18, 20.57; m/e (NH<sub>3</sub>, CI<sup>+</sup>) 142(MH<sup>+</sup>(100)), 124(28), 97(63), 84(60), 69(25).

(±) 9(ax)-Octahydroindolizidin-8-ol<sup>204</sup> (±)[261b];  $\nu_{\max}$ (soln, CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 3520(m), 2710(w), cm<sup>-1</sup>;  $\delta_{\text{H}}$  (400 MHz, CDCl<sub>3</sub>) 3.75(1H, brs, C(8)H), 3.06-2.80(3H, m, C(5)H<sub>eq</sub>, C(9)H, C(3)H<sub>eq</sub>), 2.80-2.60(1H, brs, OH), 2.04(1H, q, J = 9 Hz, C(3)H<sub>ax</sub>), 2.0-1.6(7H, m, ring protons), 1.48-1.40(1H, m, C(5)H<sub>ax</sub>), 1.32(1H, tq, J=2.69, 13.3 Hz, C(7)H<sub>ax</sub>);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 67.24, 65.48, 54.34, 52.78, 31.44, 24.89, 20.74, 19.65. (mass spectral data is not reported due to difficulties in obtaining a sample uncontaminated with the other diastereoisomer.)

(±) Octahydroindolizidine (±)[262];  $\nu_{\max}$ (soln, CH<sub>2</sub>Cl<sub>2</sub>)/cm<sup>-1</sup> 2780(m), 2700(w), 2600(m) cm<sup>-1</sup>;  $\delta_{\text{H}}$  (250 MHz, CDCl<sub>3</sub>) 3.0-2.8(2H, m, C(3)H<sub>eq</sub>, C(5)H<sub>eq</sub>), 2.08(1H, q, J=8.9 Hz, C(3)H<sub>ax</sub>), 1.98(1H, dt, J=11.2, 3.4 Hz, C(5)H<sub>ax</sub>), 1.85-0.92(11H, m, ring protons);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 64.19, 54.11, 52.90, 30.93, 30.33, 25.35, 24.38, 20.46.

(5S,8S,9R)-8-Hydroxyoctahydroindolizidine-5-carboxylic acid methyl ester [215]. A solution of [214] (500 mg, 1.15 × 10<sup>-3</sup> moles) in dry methanol (100 ml) with 5% Rh on alumina (w/w with substrate) was hydrogenated at 55 psi for 8 h. The catalyst was removed with a celite pad and the solvent was removed *in vacuo*. Water (100 ml) was added to the residue and the solution was extracted with diethyl ether (2 × 100 ml). The aqueous layer was then extracted with



dichloromethane (2 x 100 ml) and the combined dichloromethane fractions were dried ( $\text{MgSO}_4$ ) and evaporated *in vacuo* to yield [215] as a pale yellow oil as the single pure product (420 mg, 87%);  $[\alpha]_D^{20}$  -91.4 (c 1.34,  $\text{CH}_2\text{Cl}_2$ );  $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$  3526(m), 2807(s), 2734(s), 2712(w), 1741(s)  $\text{cm}^{-1}$ ;  $\delta_{\text{H}}$  (250 MHz,  $\text{CDCl}_3$ ) 3.77(1H, brs, C(8)H), 3.68(3H, s,  $\text{CO}_2\text{CH}_3$ ), 3.16(1H, dt,  $J = 2.6, 8.7$  Hz, C(3) $\text{H}_{\text{eq}}$ ), 2.79(1H, dd,  $J = 3.8, 9.7$  Hz, C(5)H), 2.57(1H, brs, OH), 2.1-1.6(9H, m, ring protons), 1.14(1H, tq,  $J = 1.6, 3.1, 8.55$  Hz, H(7) $\text{ax}$ );  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ ) 172.44, 66.56, 64.69, 52.18, 51.70, 31.21, 24.58, 23.78(2), 20.27;  $m/e$  ( $\text{NH}_3$ ,  $\text{CI}^+$ ) 200(100), 185(53), 96(13); [Found, 200.165( $\text{MH}^+$ ),  $\text{C}_{10}\text{H}_{18}\text{NO}_3$  requires 200.1282].

**(5S,9R)-Octahydroindolizidine-5-carboxylic acid methyl ester [263].** A solution of [214] (670 mg,  $3.96 \times 10^{-3}$  moles) in dry methanol (100 ml) containing  $\text{H}_2\text{SO}_4$  (6 drops) with 10% Pd on C (w/w with substrate) was hydrogenated at 45 psi for 14 h. The catalyst was removed with a celite pad and the solvent was removed *in vacuo*. Water (100 ml) was added to the residue, the solution basified ( $\text{Na}_2\text{CO}_3$ ) and extracted with diethyl ether (2 x 100 ml). The aqueous layer was then extracted with dichloromethane (2 x 100 ml) and the combined dichloromethane fractions were dried ( $\text{MgSO}_4$ ) and evaporated *in vacuo* to yield [263] as a pale yellow oil as the single pure product (520 mg, 85%);  $[\alpha]_D^{20}$  -109.4° (c = 1.592,  $\text{CHCl}_3$ );  $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$  2980(s), 2734(s), 2712(w), 1749(s)  $\text{cm}^{-1}$ ;  $\delta_{\text{H}}$  (250 MHz,  $\text{CDCl}_3$ ) 3.68(3H, s,  $\text{CO}_2\text{CH}_3$ ), 3.14(1H, dt,  $J = 2.6, 8.1$  Hz, C(3) $\text{H}_{\text{eq}}$ ), 2.80(1H, dd,  $J = 3.8, 9.7$  Hz, C(5)H), 1.86(1H, q,  $J = 8.7$  Hz, C(3) $\text{H}_{\text{ax}}$ ), 1.79-1.32(11H, m, ring protons);  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ ) 171.2, 67.12, 63.87, 60.25, 51.93, 29.80, 29.50, 20.26(2), 14.08;  $m/e$  ( $\text{NH}_3$ ,  $\text{CI}^+$ ) 184 ( $\text{MH}^+$ , 100), 124(41); [Found, 184.147( $\text{MH}^+$ ),  $\text{C}_{10}\text{H}_{18}\text{NO}_2$  requires 184.133].

**(5S,8S,9R)-8-Hydroxyoctahydroindolizidine-5-carboxylic acid ethyl ester [264].** A solution of [238] (240 mg,  $1.15 \times 10^{-3}$  moles) in dry methanol (100 ml) with 5% Rh on alumina (w/w with substrate) was hydrogenated at 55 psi for 8 h.

The catalyst was removed with a celite pad and the solvent was removed *in vacuo*. Water (100 ml) was added to the residue and extracted with diethyl ether (2 x 100 ml). The aqueous layer was then extracted with dichloromethane (2 x 100 ml) and the combined dichloromethane fractions were dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield [264] as a pale yellow oil as the single pure product (210 mg, 87%);  $[\alpha]_D^{20}$  -90.7 (c 1.6, CH<sub>2</sub>Cl<sub>2</sub>);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  3522(m), 2980(s), 2734(s), 2712(w), 1753(s) cm<sup>-1</sup>;  $\delta_{\text{H}}$  (250 MHz, CDCl<sub>3</sub>) 4.15(2H, q, J = 7.25 Hz, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 3.78(1H, brs, C(8)H), 3.19(1H, dt, J = 2.6, 8.1 Hz, C(3)H<sub>eq</sub>), 2.78(1H, dd, J = 3.8, 9.8 Hz, C(5)H), 2.64(1H, brs, OH), 2.1-1.4(10H, m, ring protons), 1.24(3H, t, J = 6.97 Hz, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 172.67, 66.72, 64.75, 60.56, 57.10, 31.27, 24.61, 23.79(2), 20.41, 14.09; m/e (NH<sub>3</sub>, CI<sup>+</sup>) 214(37), 140(100), 82(22); [Found 214.1443(MH<sup>+</sup>), C<sub>11</sub>H<sub>20</sub>NO<sub>3</sub> requires 214.1438].

(5S,9R)-Octahydroindolizidine-5-carboxylic acid ethyl ester [265]. A solution of [238] (780 mg, 3.96 x 10<sup>-3</sup> moles) in dry methanol (100 ml) with 10% Pd on C (w/w with substrate) was hydrogenated at 45 psi for 14 h. The catalyst was removed with a celite pad and the solvent was removed *in vacuo*. Water (100 ml) was added to the residue, the solution basified (Na<sub>2</sub>CO<sub>3</sub>) and extracted with diethyl ether (2 x 100 ml). The aqueous layer was then extracted with dichloromethane (2 x 100 ml) and the combined dichloromethane fractions were dried (MgSO<sub>4</sub>) and evaporated *in vacuo* to yield [265] as a pale yellow oil as the single pure product (665 mg, 85%);  $[\alpha]_D^{20}$  -80.2°(c = 1.592, CHCl<sub>3</sub>);  $\nu_{\max}(\text{neat})/\text{cm}^{-1}$  2980(s), 2734(s), 2712(w), 1745(s) cm<sup>-1</sup>;  $\delta_{\text{H}}$  (250 MHz, CDCl<sub>3</sub>) 4.13(2H, q, J = 7.2 Hz, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 3.14(1H, dt, J = 2.6, 8.1 Hz, C(3)H<sub>eq</sub>), 2.65(1H, dd, J = 3.8, 9.8 Hz, C(5)H), 1.86(1H, q, J = 8.1 Hz, C(3)H<sub>ax</sub>), 1.83-1.1(11H, m, ring protons), 1.18(3H, t, J = 7.2 Hz, CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 173.03, 67.14, 63.92, 60.35, 51.93, 29.84, 29.54, 24.06(2), 20.27, 14.08; m/e (NH<sub>3</sub>, CI<sup>+</sup>) 198(100), 124(41); [Found, 198.1494(MH<sup>+</sup>), C<sub>11</sub>H<sub>20</sub>NO<sub>2</sub> requires 198.1489].

**(5S,9R)-8-Oxoctahydroindolizidine-5-carboxylic acid methyl ester [281].** To a solution of oxalyl chloride (140 mg,  $1.1 \times 10^{-3}$  moles, 1.1 equiv) in dichloromethane (10 ml) at  $-78^{\circ}\text{C}$  under nitrogen, was added dimethyl sulfoxide (200 mg,  $2.4 \times 10^{-3}$  moles, 2.4 equiv) in dichloromethane (5 ml) over 5 min. Stirring was continued at  $-78^{\circ}\text{C}$  for 10 min followed by the addition of [215] (200 mg,  $1 \times 10^{-3}$  moles, 1 equiv) dissolved in dichloromethane (10 ml). The reaction mixture was stirred for 15 min, then triethylamine (0.7 ml,  $5 \times 10^{-2}$  moles) was added over 5 min with stirring. The cooling bath was removed and water (30 ml) was added at room temperature. Stirring was continued for 10 min and the organic layer was separated. The aqueous phase was re-extracted with dichloromethane (20 ml), the organic layers were combined and washed successively with dilute HCl (20 ml), water (20 ml), dilute sodium carbonate (20 ml) and water (20 ml). The organic layers were then dried ( $\text{MgSO}_4$ ) and evaporated to dryness to give almost colourless crude ketone [281] which was used without further purification (110 mg, 55%);  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ , 250 MHz) 3.74 (3H, s,  $\text{CO}_2\text{CH}_3$ ), 3.29 (1H, dd,  $J=9.9$ , 3.8 Hz, C(5)H), 3.21 (1H, dt,  $J=8.1$ , 2.6 Hz, C(3)H<sub>eq</sub>), 2.77 (1H, t,  $J=8.1$  Hz, C(3)H<sub>ax</sub>), 2.58-1.6 (9H, m, ring protons);  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ ) 205.48, 172.30, 69.71, 64.31, 52.42, 37.91, 28.75, 22.68, 20.45, 17.21.

**5,6,7,8-Tetrahydroindolizidine-5-carboxylic acid methyl ester [282].** A dry distillation kit, under nitrogen, was charged with [214] (200mg,  $1 \times 10^{-3}$  moles, 1 equiv) and dichloromethane (20 ml). This was heated to reflux and borane-dimethyl sulfide (0.38 ml,  $5 \times 10^{-3}$  moles, 5 equiv) was added over 5 min. Reflux was continued until *ca* 5 ml of dimethyl sulfide/dichloromethane had distilled over, (accumulation of dimethyl sulfide seems to be detrimental to the yield of the reaction). The reaction mixture was cooled to room temperature and saturated sodium carbonate (10 ml) and dichloromethane

(10 ml) were added. When the frothing had subsided, the quenched mixture was extracted with dichloromethane (2x20 ml), the organic layer was removed, washed with water (20 ml) and brine (20 ml). The organic phase was dried (MgSO<sub>4</sub>) and the solvent was removed *in vacuo* to yield a yellow oil (190 mg). Purification by chromatography (50% EtOAc:Pet ether;SiO<sub>2</sub>) gave [282] (160 mg, 80%);  $\nu_{\max}$  (soln, CHCl<sub>3</sub>)/cm<sup>-1</sup> 2955(s) 2241(m) 1751(s);  $\delta_{\text{H}}$  (250 MHz, CDCl<sub>3</sub>) 6.51 (1H, m, C(3)H), 6.16 (1H, m, C(1)H), 5.88 (1H, m, C(2)H), 4.76(1H, m, C(5)H), 3.75(3H, s, CO<sub>2</sub>Me), 2.79(2H, m, C(8)CH<sub>2</sub>), 2.25(2H, m, C(6)CH<sub>2</sub>), 1.82(2H, m, C(7)CH<sub>2</sub>);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 172.22, 128.85, 119.13, 108.22, 104.39, 57.11, 52.51, 27.24, 23.05, 18.82; *m/z* (CI, NH<sub>3</sub>) 180(MH<sup>+</sup>, 80%), 162(74), 120(100), 91(69).

### 5.3 Experimental details for Chapter 3.

(5S,9R)-(Octahydroindolizidine-5-yl)methanol [314]. To LiAlH<sub>4</sub> (64 mg, 1.7 x 10<sup>-3</sup> moles, 1.5 equiv) under nitrogen was added dry THF (10 ml) at 0°C, and the suspension was left for 10 min. To this suspension was added [265] (240 mg, 1.13 x 10<sup>-3</sup> moles) in THF (5 ml). After 1.5 h at 5-10°C, KOH (50 mg) and water (5 ml) were added and the solution was left for 15 min. The aqueous solution was then decanted and extracted with diethyl ether (3 x 20 ml). The combined organic layers were then washed with brine (20 ml), dried (MgSO<sub>4</sub>) and the solvent removed *in vacuo* to yield [314] as a colourless oil (120 mg, 69%);  $[\alpha]_{\text{D}}^{20}$  -78.4 (c 1.08, CH<sub>2</sub>Cl<sub>2</sub>);  $\nu_{\max}$ (neat)/cm<sup>-1</sup> 3387(m), 2718(s);  $\delta_{\text{H}}$  (250 MHz, CD<sub>3</sub>OD) 3.61(1H, dd, *J* = 11.1, 4.8 Hz, C(10)H), 3.38(1H, dd, *J* = 11.1, 5.9 Hz C(10)H'), 3.17(1H, dt, *J* = 8.4, 2.6 Hz, C(3)H<sub>eq</sub>), 2.03(1H, q, *J* = 8.4 Hz, C(3)H<sub>ax</sub>), 2.05-1.07(13H, m, ring protons);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 65.21, 65.03, 64.25, 51.13, 29.73, 29.15, 28.00, 23.66, 19.89; *m/e* (NH<sub>3</sub>, CI<sup>+</sup>) 156(100), 124(13); [Found, 156.1388(MH<sup>+</sup>), C<sub>9</sub>H<sub>18</sub>NO requires 156.1384].

**(5S,9R)-(Octahydroindolizidine-5-yl)methyl-O-*p*-toluene sulfonate [315].** To a solution of [314] (90 mg,  $5.77 \times 10^{-4}$  moles) in dry  $\text{CH}_2\text{Cl}_2$  (10 ml) under an atmosphere of nitrogen at  $-78^\circ\text{C}$  was added  $n\text{BuLi}$  (0.27 ml,  $6.92 \times 10^{-4}$  moles, 1.2 equiv). This solution was left for 10 min in which time it was allowed to warm to  $-20^\circ\text{C}$ . Tosyl chloride (217 mg,  $1.15 \times 10^{-3}$  moles, 2.0 equiv) was added portionwise and the reaction mixture was allowed to attain room temperature overnight. Water (10 ml) was then added to the solution, the aqueous layer was separated and extracted with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 10$  ml). The combined organic layers were then dried ( $\text{MgSO}_4$ ) and the solvent was removed *in vacuo* to yield an off white solid (200 mg). Purification by column chromatography (1:1 EtOAc:Pet ether,  $\text{SiO}_2$ ) yielded [315] as a colourless oil (36 mg, 26%);  $[\alpha]_D^{20} +20.4$  (c 1.73,  $\text{CH}_2\text{Cl}_2$ );  $\nu_{\text{max}}(\text{neat})$  2718(s);  $\delta_{\text{H}}$  (250 MHz,  $\text{CD}_3\text{OD}$ ) 7.69(2H, d,  $J=7.8$  Hz, ArH), 7.10(2H, d,  $J=7.8$  Hz, ArH), 3.61(1H, dd,  $J=11.1, 4.9$  Hz, C(10)H), 3.42(1H, dd,  $J=11.1, 5.9$  Hz C(10)H'), 3.17(1H, dt,  $J=8.4, 2.6$  Hz, C(3) $\text{H}_{\text{eq}}$ ), 2.45(3H, s, ArCH<sub>3</sub>), 2.03(1H, q,  $J=8.4$  Hz, C(3) $\text{H}_{\text{ax}}$ ), 2.05-1.11(12H, m, ring protons);  $\delta_{\text{C}}$  ( $\text{CDCl}_3$ ) 147.0, 139.86, 129.59, 127.02, 65.21, 65.03, 64.25, 52.10, 51.13, 29.73, 29.15, 28.00, 23.66, 19.89.

**Reaction of (5S,9R)-(octahydroindolizidine-5-yl)-methyl-O-*p*-toluene sulfonate [315] with lithiocuprates, (general procedure).** To a suspension of copper (I)-iodide (4 equiv) in dry  $\text{Et}_2\text{O}$  (20 ml) under an atmosphere of nitrogen at  $-20^\circ\text{C}$  was added a solution of alkyl lithium in  $\text{Et}_2\text{O}$  (8 equiv) portionwise to give a dark yellow suspension after half the addition and a black solution after the addition was complete. This solution was transferred by cannular into a solution of [315] (1 equiv) in dry THF (5 ml), under an atmosphere of nitrogen at  $-20^\circ\text{C}$ . The solution was left at  $-20^\circ\text{C}$  for 8 h and then left to attain room temperature overnight. The reaction mixture was quenched with saturated aqueous  $\text{NH}_4\text{Cl}$  (10 ml) and extracted with  $\text{Et}_2\text{O}$  ( $2 \times$

10 ml). The combined organic layers were then dried ( $\text{MgSO}_4$ ) and the solvent removed *in vacuo* to yield only unreacted starting material.

**Reaction of (5S,9R)-(octahydroindolizidine-5-yl)methyl-O-p-toluene sulfonate [315] with Grignard reagents. (general procedure).** To a solution of [315] (1 equiv) in dry THF (10 ml) under an atmosphere of nitrogen, at  $-20^\circ\text{C}$  was added a commercially available solution of the Grignard reagent in  $\text{Et}_2\text{O}$  (4 equiv) over 10 min. The solution was maintained at  $-20^\circ\text{C}$  for 5 h before being allowed to attain room temperature overnight. The reaction mixture was then quenched with water (5 ml) and extracted with  $\text{Et}_2\text{O}$  ( $2 \times 10$  ml). The organic extracts were combined, dried ( $\text{MgSO}_4$ ) and the solvent removed *in vacuo* to yield only unreacted starting material.

**8-Oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid methyl ester [214].** A solution of 8-oxo-5,6,7,8-tetrahydroindolizidine-5-carboxylic acid ethyl ester [238] (100 mg,  $4.83 \times 10^{-4}$  moles) in methanol (5 ml) containing  $\text{H}_2\text{SO}_4$  (6 drops) was refluxed for 3 h. The solution was then cooled to room temperature and diluted with water (20 ml). This solution was then extracted with  $\text{CH}_2\text{Cl}_2$  ( $3 \times 10$  ml), the organic layers dried ( $\text{MgSO}_4$ ) and the solvent was removed *in vacuo* to yield a pale brown oil (134 mg). The crude product was then purified by column chromatography ( $\text{Et}_2\text{O}$ ,  $\text{SiO}_2$ ) to yield material identical to [214] (68mg, 74%).

**(5S,8S,9R)-5-(methoxycarbonyl)octahydroindolizidine-8-yl-O-p-toluene sulfonate [308].** To a suspension of sodium hydride (60% dispersion, 42 mg,  $1.06 \times 10^{-3}$  moles, 1.5 eq) in THF (10 ml) was added a solution of (5S,8S,9R)-5-methoxycarbonyl-8-hydroxyindolizidine [215] (160 mg) in THF (1 ml) at  $-78^\circ\text{C}$ . After 10 min tosyl chloride (200 mg,  $1.06 \times 10^{-3}$  moles, 1.5 eq) was added portionwise and the resulting solution was left stirring to attain room

temperature for 12 h. Water (5 ml) was added carefully and the resulting solution was extracted with dichloromethane (3 x 10 ml). The organic layers were combined, dried (MgSO<sub>4</sub>) and the solvent was removed *in vacuo* to yield a brown oil (140 mg). Purification by column chromatography (50% ethyl acetate : pet ether ; SiO<sub>2</sub>) gave [308] as a colourless oil (74mg, 20%);  $\nu_{\max}(\text{neat})$  2718(s);  $\delta_{\text{H}}$  (250 MHz, CDCl<sub>3</sub>) 7.69(2H, d,  $J=7.8$  Hz, ArH), 7.10(2H, d,  $J=7.8$  Hz, ArH), 3.86(1H, br s, C(8)H), 3.69(3H, s, CO<sub>2</sub>Me), 3.35(1H, dist t,  $J=8.7$  Hz, C(3)H<sub>eq</sub>), 3.12(1H, dd,  $J=3.7, 9.8$  Hz, C(5)H), 2.45(3H, s, ArCH<sub>3</sub>), 2.21(1H, m, C(9)H), 2.05-1.67(9H, m, ring protons);  $\delta_{\text{C}}$  (CDCl<sub>3</sub>) 171.84, 146.74, 139.73, 129.57, 126.98, 67.37, 64.37, 52.18, 52.05, 30.94, 24.29, 23.31(x2), 21.68, 20.13.

**(5S,8S,9R)-8-[(1,1-dimethylethyl)dimethylsiloxy]octahydroindolizidine-5-carboxylic acid methyl ester [311]** To a solution of [215] (60 mg,  $3.02 \times 10^{-4}$  moles) in DMF (1 ml) containing imidazole (21 mg,  $3.3 \times 10^{-4}$  moles, 1.1 equiv) at 0°C was added tBDMSCl (50 mg,  $3.3 \times 10^{-4}$  moles, 1.1 equiv) portionwise over 10 min. The solution was then heated under reflux for 4 h, cooled and diluted with water (10 ml), and CH<sub>2</sub>Cl<sub>2</sub> (10 ml) was added. The aqueous layer was then separated and extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 x 10 ml). The combined organic layers were then dried (MgSO<sub>4</sub>) and the solvent was removed *in vacuo* to yield a pale brown oil (70 mg). Purification by chromatography(50% ethyl acetate:pet ether;SiO<sub>2</sub>) gave [311] as a colourless oil (10 mg, 11%);  $[\alpha]_{\text{D}}^{20}$  -24.7(c 1.64, CH<sub>2</sub>Cl<sub>2</sub>);  $\nu_{\max}(\text{neat})$  2718(s);  $\delta_{\text{H}}$  (250 MHz, CDCl<sub>3</sub>) 3.91(1H, br s, C(8)H), 3.72(3H, s, CO<sub>2</sub>Me), 3.21(1H, dist t,  $J=6.7$  Hz, C(3)H<sub>eq</sub>), 2.76(1H, dd,  $J=11.2, 2.9$  Hz, C(5)H), 2.1-1.4(9H, m, ring protons), 1.25(1H, m, C(7)H<sub>eq</sub>), 0.905, (9H, s, <sup>t</sup>Bu group), 0.05(6H, s, Si(CH<sub>3</sub>)<sub>2</sub>).

**(5S,8S,9R)-8-O-Methoxymethyloctahydroindolizidine-5-carboxylic acid methyl ester [312].** To a solution of [215] (90 mg,  $4.5 \times 10^{-4}$  moles) in DMF (1 ml) containing (iPr)<sub>2</sub>NEt (1 ml) at 0°C was added methoxymethyl chloride (0.05

ml,  $6.78 \times 10^{-4}$  moles, 1.5 equiv) over 10 min. The solution was left at 0°C for 1 h and then allowed to attain room temperature overnight. Water (10 ml), and  $\text{CH}_2\text{Cl}_2$  (10 ml) were then added. The aqueous layer was then separated and extracted with  $\text{CH}_2\text{Cl}_2$  ( $2 \times 10$  ml). The combined organic layers were then dried ( $\text{MgSO}_4$ ) and the solvent was removed *in vacuo* to yield a pale brown oil. Purification by chromatography (80% EtOAc:20% Pet ether;  $\text{SiO}_2$ ) gave [312] as a colourless oil (55mg, 30%);  $\delta_{\text{H}}$  (250 MHz,  $\text{CDCl}_3$ ) 4.77(1H, d,  $J=6.97$  Hz,  $-\text{O}-\text{CH}_2-\text{O}-$ ), 4.62(1H, d,  $J=6.97$  Hz,  $-\text{O}-\text{CH}_2-\text{O}-$ ), 3.80(1H, br s, C(8)H), 3.71(3H, s,  $\text{CO}_2\text{Me}$ ), 3.37(3H, s,  $\text{OCH}_3$ ), 3.22(1H, dist t,  $J=6.97$  Hz, C(3) $\text{H}_{\text{ax}}$ ), 2.78(1H, dd,  $J=11.47, 2.6$  Hz, C(5)H), 2.1-1.6(10H, m, ring protons).

**8-Oxa-2-aza-tricyclo-[5.2.2.0]-2,6-undecan-9-one [313].** To a suspension of sodium hydride (60% dispersion, 31 mg,  $7.6 \times 10^{-4}$  moles, 1.2 eq) in THF (5 ml) was added a solution of (5S,8S,9R)-5-ethoxycarbonyl-8-hydroxyindolizidine [264] (160 mg) in THF (1 ml) at 0°C. After 10 min,  $\text{TMSCl}$  (0.1 ml,  $7.6 \times 10^{-4}$  moles, 1.2 eq) was added and the resulting solution was left stirring to attain room temperature for 12 h. Water (5 ml) was added carefully and the resulting solution was extracted with dichloromethane ( $3 \times 10$  ml). The organic layers were combined, dried ( $\text{MgSO}_4$ ) and the solvent was removed *in vacuo* to yield a brown oil (140 mg). Purification by column chromatography ( $\text{Et}_2\text{O}$ ,  $\text{Al}_2\text{O}_3$ ) yielded a white solid (50 mg, 39%);  $[\alpha]_{\text{D}}^{20} +6.77^\circ$  ( $c = 1.0$ ,  $\text{CHCl}_3$ );  $\nu_{\text{max}}(\text{neat})/\text{cm}^{-1}$  2876(s), 1751(s)  $\text{cm}^{-1}$ ;  $\delta_{\text{H}}$  (400 MHz,  $\text{CDCl}_3$ ) 4.57(1H, s, C(8)H), 3.34(1H, m, C(5)H), 3.27(1H, t,  $J = 8$  Hz, C(9)H), 3.14(1H, t,  $J = 8$  Hz, C(3) $\text{H}_{\text{ax}}$ ), 2.53(1H, m, C(3) $\text{H}_{\text{eq}}$ ), 2.10(1H, m, C(6) $\text{H}_{\text{ax}}$ ), 2.0-1.6(7H, m, ring protons);  $\delta_{\text{C}}$  (100 MHz,  $\text{CDCl}_3$ ) 173.34, 76.61, 60.69, 56.65, 53.37, 27.75, 24.81, 24.06, 23.50;  $m/e$  ( $\text{NH}_3$ ,  $\text{CI}^+$ ) 168(93), 140(46), 123(100), 110(45), 96(78), 83(38), 49(55), 41(35), [Found, 167.0946( $\text{MH}^+$ )  $\text{C}_9\text{H}_{14}\text{NO}_2$  requires 168.1021].

## 5.4 Experimental details for Chapter 4.



***N*-{3-[Acetyl-(4-acetylaminobutyl)amino]propyl}acetamide<sup>205</sup> [380].** To a solution of *N*-1-(3-amino-propyl)-1,4-diaminobutane [363] (1.01g,  $6.9 \times 10^{-3}$  moles) in toluene (20 ml) at 0°C under nitrogen, was added acetic anhydride (6.6 ml,  $6.9 \times 10^{-2}$  moles) over 30 min. The solution was left overnight and the solvent and excess acetic anhydride were removed *in vacuo* to yield a pale yellow oil. Purification by column chromatography (MeOH : CH<sub>2</sub>Cl<sub>2</sub>, 1 : 10, SiO<sub>2</sub>) yielded [380] as a colourless oil (1.66g, 88%);  $\delta^1\text{H}$  (400 MHz, CD<sub>3</sub>OD), [H = major isomer, H' = minor isomer] 3.41(4H, m, H, H'(3)), 3.38(4H, m, H, H'(5)), 3.25(4H, m, H'(1,7)), 3.21(4H, m, H(1,7)), 2.15(3H, s, N(4)Ac), 2.14(3H, s, N'(4)Ac), 2.00(3H, s, N(1)Ac), 1.99(3H, s, N(8)Ac), 1.85(2H, m, H'(2)H), 1.78(2H, m, H(2)), 1.67(4H, m, H(6,7)), 1.56, (4H, m, H'(6,7));  $\delta^1\text{C}$  (CD<sub>3</sub>OD) 171.1, 170.5, 48.4, 47.0, 45.3, 42.5, 38.6, 37.0, 36.1, 29.0, 27.7, 27.4, 27.1, 26.5, 25.8, 24.8, 23.3, 23.2, 23.0, 21.4 ; m/e (NH<sub>3</sub>, CI<sup>+</sup>) 272(74%).

**Tris(benzyloxycarbonyl)-*N*-1-(3-aminopropyl)butane-1,4-diamine<sup>186</sup> [381].** To a solution of *N*-1-(3-aminopropyl)-1,4-diaminobutane [363] (0.82g,  $5.7 \times 10^{-3}$  moles) in saturated aqueous Na<sub>2</sub>CO<sub>3</sub> (20 ml) at 0°C under nitrogen was added benzyl chloroformate (2.7 ml, 0.019 moles) over 30 min. The solution was left overnight. The resulting biphasic system was then extracted with ethyl acetate (3 x 20 ml) and the organic layers were then combined, dried with MgSO<sub>4</sub> and the solvent removed *in vacuo* to yield a pale yellow oil. Purification by column chromatography (MeOH : CH<sub>2</sub>Cl<sub>2</sub>, 1 : 10, SiO<sub>2</sub>) yielded [381] as a pale yellow oil (2.18g, 70%);  $\delta^1\text{H}$  (400 MHz, CDCl<sub>3</sub>); 7.21(15H, m, N(1,4,8)Ar), 4.97(2H, s, N(4)CH<sub>2</sub>), 4.94(4H, s, N(1,8)CH<sub>2</sub>), 3.19(4H, m, C(1,3)CH<sub>2</sub>), 2.99((4H, m, C(5,8)CH<sub>2</sub>), 1.60(2H, qn, J = 6.9 Hz, C(2)CH<sub>2</sub>), 1.37(4H, m, C(6,7)CH<sub>2</sub>);  $\delta^1\text{C}$  (CD<sub>3</sub>OD) 173.46, 173.35, 173.25, 173.06, 49.69, 47.62, 46.51, 44.59, 40.38, 40.10, 39.95, 38.15, 37.90, 29.42, 28.32, 27.57, 27.51, 26.96, 25.82, 22.50, 22.46, 22.42, 21.29, 21.25 ; m/e (NH<sub>3</sub>, CI<sup>+</sup>) 548(MH<sup>+</sup>, 74%).

**N-(4-Aminobutyl)acetamide**<sup>185</sup> [379]. To a solution of butane-1,4-diamine [365](5g, 0.057 moles) in glacial acetic acid (35 ml) at 60°C was added a mixture of acetic anhydride (4.5 ml, 0.048 moles) and glacial acetic acid (5 ml) over *ca* 3 h. The solution was left overnight and excess acetic anhydride and the solvent were removed *in vacuo*. The residue was dissolved in hot water (40 ml), cooled to 0°C and adjusted to pH < 1 with 6M HCl (10 ml). After evaporation to dryness *in vacuo* the resulting white solid was extracted with hot propan-2-ol (4 x 20 ml). The combined extracts were concentrated *in vacuo* and cooled to -10°C. The resulting crystals were collected and recrystallised from propan-2-ol yielding white crystals of [379] (0.85g, 12%); mp 137-140°C (lit mp 136-139°C);  $\delta^1\text{H}$  (250 MHz,  $\text{CD}_3\text{OD}$ ) 4.37(2H, t, J=6.4 Hz,  $\text{CH}_2\text{NHAc}$ ), 2.99(2H, t, J=7.6 Hz  $\text{CH}_2\text{NH}_2$ ), 1.99(3H, s,  $\text{CH}_3$ ), 1.56(4H, m, 2 x  $\text{CH}_2$ );  $\delta\text{C}$  ( $\text{CD}_3\text{OD}$ ) 170.2, 40.36, 39.56, 27.36, 25.86, 22.60; m/e ( $\text{NH}_3$ ,  $\text{CI}^+$ ) 131(60%).

**N, N'-Bis(benzyloxycarbonyl)butane-1,4-diamine** [378]. To a solution of butane-1,4-diamine [365](1.65g, 0.19 moles) in saturated  $\text{Na}_2\text{CO}_3$  (100 ml) at 0°C under nitrogen was added benzyl chloroformate (7 ml, 0.4 moles) over 30 min. The reaction mixture was left overnight and water (20 ml) was added. The pH of the solution was adjusted to 7 producing a white solid which was collected and recrystallised from methanol yielding colourless crystals of [378] (4.39g, 66%); mp 137-139°C;  $\delta^1\text{H}$  (250 MHz,  $\text{CDCl}_3$ ) 7.34(10H, m, Ar), 5.08(4H, s,  $\text{CH}_2\text{-Ar}$ ), 4.88(2H, brs,  $\text{NH}$ ), 3.18(4H, m,  $\text{NH-CH}_2$ ), 1.51(4H, m,  $\text{NH-CH}_2\text{CH}_2$ );  $\delta\text{C}$  ( $\text{CDCl}_3$ ) 173.78, 156.19, 136.35, 128.32, 127.91, 66.46, 40.44, 27.03; m/e ( $\text{NH}_3$ ,  $\text{CI}^+$ ) 357(72%); m/e ( $\text{NH}_3$ ,  $\text{CI}^+$ ) [Found, 357.1816(MH+)  $\text{C}_{20}\text{H}_{27}\text{N}_2\text{O}_4$  requires 357.1808].

**Lypholysed Enzymes (general procedure).** To the enzyme (100 mg) was added buffer (10ml) and the resulting solution stirred at room temperature for 3 h. The solution was then frozen in liquid nitrogen and placed on a freeze-dryer

over night or until a dry powder was obtained. This was then used in the procedures as 'biocatalyst'. Pre-lypholysed enzyme was used in all procedures to allow for direct comparison.

### **Enzymatic hydrolyses (general procedures).**

#### **Aqueous buffered solutions.**

To substrate (50 mg) in solvent (10 ml) was added biocatalyst (10 mg). See Tables 4, 5. The reaction was monitored by TLC over a period of 4-10 days, using MeOH:CH<sub>2</sub>Cl<sub>2</sub> (1:3) as the solvent system and visualising the compounds with either iodine vapour or ninhydrin. Samples were taken at intervals and partitioned between water and ethyl acetate and both layers analysed by TLC.

#### **Co-solvent systems.**

To substrate (50 mg) in solvent (10 ml) was added biocatalyst (10 mg). See Tables 4, 5. The reaction was monitored by TLC over a period of 4-10 days, using MeOH:CH<sub>2</sub>Cl<sub>2</sub> (1:3) as the solvent system and visualising the compounds with either iodine vapour or ninhydrin. Samples were taken at intervals and partitioned between water and ethyl acetate and both layers analysed by TLC.

#### **Low Water systems.**

To substrate (50 mg) in solvent (10 ml) was added biocatalyst (10 mg). See Tables 4, 5. The reaction was monitored by TLC over a period of 4-10 days, using MeOH:CH<sub>2</sub>Cl<sub>2</sub> (1:3) as the solvent system and visualising the compounds with either iodine vapour or ninhydrin. Samples were taken at intervals and analysed directly by TLC.

**Bis-(phenyl acetyl)-butane-1,4-diamine [388]**

To butane-1,4-diamine (1.11g, 0.013 moles) in toluene (20 ml) under nitrogen was added phenylacetyl chloride (3.68 ml, 0.03 moles) over 10 min. This was left for 36 hours. Removal of the solvent *in vacuo* yielded a white solid (3.97g) which was recrystallised from ethanol to give [388] as a white crystalline solid (2.94g, 72%); mp 178-180°C;  $\nu_{\max}(\text{nujol})/\text{cm}^{-1}$  3254(m), 1628(m); (Found C, 73.72; H, 7.42; N, 8.73%;  $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_2$  requires C, 74.0; H, 7.46; N, 8.6%);  $\delta^1\text{H}$  (250 MHz,  $\text{CDCl}_3/\text{CD}_4\text{OD}$ ) 7.13(10H, m, Ar), 3.42(4H, s,  $\text{CH}_2\text{Ar}$ ), 3.06(4H, m,  $\text{CH}_2\text{NH}$ ), 1.32(4H, m,  $\text{CH}_2\text{CH}_2\text{NH}$ );  $\delta_{\text{C}}(\text{CDCl}_3)$  173.88, 156.29, 136.44, 128.41, 128.00, 66.56, 40.54, 27.12; m/e ( $\text{NH}_3$ ,  $\text{CI}^+$ ) 325( $\text{MH}^+$ , 100%), 223(18), 91(27).

**Bis-(phenyl-isothiocyano)-butane-1,4-diamine [389]**

To aqueous ethanol (100 ml, 4:5[v/v]) and butane-1,4-diamine (0.13g,  $1.47 \times 10^{-3}$ ) was added phenyl isothiocyanate (2.4 ml, 0.02 moles) under nitrogen. After 20 min the resulting white solid was filtered off and washed with cold ethanol. This yielded [389] as a white crystalline solid (0.347g, 66%); mp 156-158°C;  $\nu_{\max}(\text{nujol})/\text{cm}^{-1}$  3412(m), 1593(m), 1542(s); (Found C, 60.35; H, 6.16; N, 15.42%;  $\text{C}_{18}\text{H}_{22}\text{N}_4\text{S}_2$  requires C, 60.30; H, 6.20; N, 15.6%);  $\delta^1\text{H}$  (250 MHz,  $(\text{CD}_3)_2\text{CO}$ ) 7.37(10H, m, Ar), 3.65(4H, m,  $\text{CH}_2\text{NH}$ ), 3.16(2H,  $\text{ArNH}$ ), 2.93(2H,  $\text{NH}$ ), 1.68(4H, m,  $\text{CH}_2\text{CH}_2\text{NH}$ );  $\delta_{\text{C}}((\text{CD}_3)_2\text{CO})$  182.27, 162.67, 129.83, 125.73, 124.72, 44.77, 27.07.

**N-(5-aminopentyl)-3-aminopropionitrile<sup>201</sup> [390].** Acrylonitrile (1.29 ml, 0.0253 moles) was added dropwise over 30 min to a solution of 1,4-diaminopentane (2.3 ml, 0.0196 moles) in a mixture of ethanol (30 ml) and water (20 ml) and left over night. The solution was heated over a steam bath for one hour, cooled, acidified to pH 3 with dilute HCl, and the solvent removed *in vacuo* to yield a white solid. Recrystallization from ethanol

yielded [390] as white crystals of the HCl salt (0.727g, 24%); mp 218-221°C (lit mp 216-219°C);  $\nu_{\max}(\text{nujol})/\text{cm}^{-1}$  3388(m), 2256(s);  $\delta^1\text{H}$  (300 MHz,  $\text{CD}_3\text{OD}$ ) 3.37(2H, dist t,  $J=7.2$  Hz,  $\text{CH}_2\text{CN}$ ), 3.09(2H, dist t,  $J=7.2$  Hz,  $\text{NHCH}_2\text{CH}_2\text{CN}$ ), 3.01(2H, dist t,  $J=7.2$  Hz,  $\text{CH}_2\text{NHCH}_2\text{CH}_2\text{CN}$ ), 2.98(2H, dist t,  $J=7.2$  Hz,  $\text{CH}_2\text{NH}_2$ ), 1.76(4H, m, C(7,8) $\text{CH}_2$ ), 1.50(2H, m, C(6) $\text{CH}_2$ );  $\delta^1\text{C}$  ( $\text{CD}_3\text{OD}$ ) 116.35, 43.11, 39.17, 27.82, 26.69, 25.34, 23.14, 14.44; m/e ( $\text{NH}_3$ ,  $\text{CI}^+$ ) 156( $\text{MH}^+$ )[Found, 156.1499 ( $\text{MH}^+$ )  $\text{C}_8\text{H}_{18}\text{N}_3$  requires 156.1497].

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